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นางสาวมะลิวัลย์ คุตะโค

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COMPOSITION AND ROLE OF MICROORGANISMS ON NITROGEN CYCLE IN AQUACULTURE POND

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Environmental Science
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Thesis Title	COMPOSITION AND ROLE OF MICROORGANISMS ON NITROGEN CYCLE IN AQUACULTURE POND
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Field of Study	Environmental Science
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มะลิวัลย์ คุตะโค: องค์ประกอบและบทบาทของจุลินทรีย์ต่อวัฏจักรไนโตรเจนในบ่อเลี้ยงสัตว์น้ำ. (COMPOSITION AND ROLE OF MICROORGANISMS ON NITROGEN CYCLE IN AQUACULTURE POND) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร. เปี่ยมศักดิ์ เมนะเศวต, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. สรวิศ เผ่าทองศุข. 245 หน้า.

งานวิจัยนี้ได้ศึกษากระบวนการบำบัดแอมโมเนียที่เกิดจากการทำงานของจุลินทรีย์ในดินตะกอนจากบ่อเลี้ยงกุ้งขาว (*Litopenaeus vannamei*) การทดลองแรกได้ทดลองในสภาวะห้องปฏิบัติการ โดยเก็บดินตะกอนจากบ่อเลี้ยงกุ้งในจังหวัดปทุมธานี จากนั้นบรรจุลงในภาชนะรูปทรงกระบอก เติมน้ำทะเลและให้อากาศโดยพ่นผ่านหัวทราย ตรวจวัดความเข้มข้นของแอมโมเนีย ไนไตรต์และไนเตรตในน้ำและดินตะกอน รวมทั้งตรวจวัดการเปลี่ยนแปลงความหลากหลายของแบคทีเรียในดินตะกอนโดยใช้เทคนิค PCR-DGGE วิเคราะห์ชิ้นส่วนของ 16S rRNA gene ผลการทดลองพบว่าการเปลี่ยนความเค็มน้ำจาก 5 เป็น 20 พีเอสยู อย่างรวดเร็วไม่มีผลต่อกระบวนการแอมโมนิฟิเคชัน แต่กลับมีผลยับยั้งกระบวนการดีไนตริฟิเคชัน แบคทีเรียชนิดเด่น *Marinobacter* sp. และ *Psuedomonas* sp. ไม่ปรากฏอีกหลังจากที่มีการเปลี่ยนความเค็ม เมื่อบ่มด้วยค่าการละลายของออกซิเจนในน้ำต่ำกว่า 2.5 มก./ล. พบว่ากระบวนการไนตริฟิเคชันถูกยับยั้งและยังส่งผลให้ความหลากหลายของแบคทีเรียในดินตะกอนลดลง การเติมโซเดียมคาร์บอเนตสามารถเร่งกระบวนการแอมโมเนียออกซิเดชัน ไนไตรต์ออกซิเดชันและดีไนตริฟิเคชันได้ ในขณะที่การเติมเมทานอลกลับส่งผลเสียต่อกระบวนการไนตริฟิเคชัน และผลจากการวิเคราะห์ DGGE พบว่าดีไนตริไฟอิงแบคทีเรีย *Vibrio* sp., *Psuedomonas* sp., *Planococcus* sp., *Streptomyces* sp. และ *Thioalcalovibrio* sp. ถูกกระตุ้นหลังจากที่มีการเติมเมทานอล เมื่อบ่มในสภาวะที่มีแสงพบว่าการบำบัดแอมโมเนียเกิดจากการออกซิไดซ์แอมโมเนียโดยการทำงานของแบคทีเรียและการนำแอมโมเนียเข้าสู่เซลล์ของแพลงก์ตอนพืช อย่างไรก็ตาม กระบวนการหลักในการบำบัดไนโตรเจนเกิดจากกระบวนการไนตริฟิเคชันและดีไนตริฟิเคชันโดยการทำงานของแบคทีเรียมากกว่ากระบวนที่เกิดจากการทำงานของแพลงก์ตอนพืช ส่วนการตากดินมีผลอย่างมากในการยับยั้งกระบวนการไนตริฟิเคชัน

การทดลองที่สองได้ทำการศึกษาในถังเลี้ยงกุ้งจำลองกลางแจ้งซึ่งเป็นถังพลาสติกขนาด 500 ลิตร บรรจุดินตะกอนจากบ่อเลี้ยงกุ้งและเติมน้ำเค็ม 20 พีเอสยู ปริมาตร 450 ลิตร มีการให้อากาศตลอดเวลาและวางถังในที่กลางแจ้ง ชุดการทดลองประกอบด้วย ถังควบคุมที่บรรจุด้วยดินเปียกและถังทดลองที่บรรจุด้วยดินที่ตากแดด ผลการทดลองพบว่าการตากแดดจะยับยั้งกระบวนการไนตริฟิเคชันเพราะมีแอมโมเนียและไนไตรต์สะสมในถังทดลอง แต่อย่างไรก็ตามแอมโมเนียและไนไตรต์ในถังทดลองกลางแจ้งนี้มีความเข้มข้นต่ำกว่าในภาชนะที่ทดลองในห้องปฏิบัติการ ผลจากการวิเคราะห์ด้วย DGGE ชี้ให้เห็นว่าในถังทดลองจะไม่พบแอมโมเนียออกซิไดซิแบคทีเรีย *Nitrosomonas* sp. แต่หลังจากนั้น 3 สัปดาห์ กลับตรวจพบ *Nitrosomonas* sp. เพราะสามารถปรับตัวให้เข้ากับสภาพแวดล้อมใหม่ได้ หลังจากที่ถังเลี้ยงกุ้งจำลองมีการปรับสภาพแล้วได้ทดลองเติมสารละลายแอมโมเนียมคลอไรด์เป็นตัวแทนของของเสียที่กุ้งปลดปล่อยออกสู่บ่อเลี้ยง เพื่อทดสอบอัตราการบำบัดแอมโมเนียในถังเลี้ยงกุ้ง พบว่าที่อัตราการเติมแอมโมเนียเท่ากับ 0.2 มก.ไนโตรเจน/ล./วัน ถังเลี้ยงกุ้งจำลองสามารถบำบัดแอมโมเนียได้หมดภายในเวลา 1 วัน ผลการทดลองนี้มีอัตราการบำบัดแอมโมเนียในช่วง 0.21 ถึง 0.28 มก.ไนโตรเจน/ตร.ม./วัน หรือมีค่าเท่ากับการเลี้ยงกุ้งที่มีน้ำหนัก 10 ก. ด้วยความหนาแน่น 26 ตัว/ตร.ม.

สาขาวิชา วิทยาศาสตร์สิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

4889668920 : MAJOR ENVIRONMENTAL SCIENCE
KEYWORDS : NITROGEN CYCLE / AQUACULTURE POND / SEDIMENT / MICROORGANISM DIVERSITY / PCR-DGGE

MALIWAN KUTAKO : COMPOSITION AND ROLE OF MICROORGANISMS ON NITROGEN CYCLE IN AQUACULTURE POND. ADVISOR : PROF. PIAMSAK MENASVETA, PH.D., CO-ADVISOR : SORAWIT POWTONGSOOK, PH.D., 245 pp.

This study investigated nitrogen treatment processes by natural microorganisms in sediment from white shrimp (*Litopenaeus vanamei*) pond. The first part of this study was conducted under laboratory condition. Sediment from shrimp pond in Pathum Thani Province was packed in sediment chamber, filled with seawater, and aerated using air stone. Concentrations of ammonia, nitrite, and nitrate in water column and in sediment were monitored. Changes of bacterial diversity in the sediment were examined by PCR-DGGE analysis of 16S rRNA gene. It was found that rapid changing of salinity from 5 to 20 PSU had no effect on ammonia oxidation process while denitrification was somewhat inhibited. The dominant bacterial species such as *Marinobacter* and *Psuedomonas* disappeared after salinity changed. Low DO concentration (2.5 mg/L) significantly inhibited nitrification process and also slightly decreased the diversity of bacteria in the sediment. Addition of $NaHCO_3$ in the sediment chambers accelerated the nitrogen conversion by ammonia oxidation, nitrite oxidation and denitrification processes. Methanol addition had a negative effect on nitrification process in the sediment chamber. Results from DGGE analysis suggested that denitrifying bacteria *Vibrio* sp., *Psuedomonas* sp., *Planococcus* sp., *Streptomyces* sp. and *Thioalcalovibrio* sp. were stimulated after methanol addition. When the sediment chamber was illuminated, ammonia removal was accomplished by a combination of ammonia oxidizing by bacteria and ammonia uptake by phytoplankton. However, the major role of nitrogen removal in the sediment chambers was through bacterial nitrification and denitrification processes rather than photosynthetic microorganisms. Soil desiccation by sun drying strongly inhibited nitrification process in both ammonia oxidation and nitrite oxidation steps in the sediment chambers.

The second part of this study was carried out in the outdoor artificial shrimp pond. Artificial shrimp pond was 500 L plastic tank packed with sediment from shrimp pond and filled with 450 L of 20 PSU seawater. The tanks were continuously aerated. The experiment consisted of control tank packed with wet untreated sediment and treatment tank packed with sun-dried sediment. It was found that sun drying inhibited nitrification process in the tank and peaks of ammonia and nitrite was found in treatment tank. However, peaks of ammonia and nitrite were lower than that found in the sediment chamber because of phytoplankton uptake. DGGE analysis illustrated that ammonia oxidizing bacteria (*Nitrosomonas* sp.) was disappeared from treatment tank but it was recovered after acclimation for three weeks. After pond acclimation, simulation of shrimp excretion by repeat addition of ammonium chloride into the artificial shrimp pond was performed and ammonia removal rate was examined. It was found that at the ammonia loading rate of 0.2 mg-N/L/day, the pond had a capability to remove all ammonia within one day. This was equal to the ammonia removal rate of 0.21-0.28 mg-N/m^2/day and this ammonia loading was equal to the cultivation of 10 g shrimp at a density of 26 shrimp/m^2.

Field of Study : Environmental Science Student's Signature Maliwan Kutako

Academic Year : 2008 Advisor's Signature

Co-Advisor's Signature

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LIST OF ABBREVIATIONS

AOB	Ammonia oxidizing bacteria
AOAC	Association of Official Analytical Chemists
ABS	Absorbance
APHA	American Public Health Association
BOD	Biological oxygen demand
°C	Degree Celsius
cm	Centimeter
CFB	Cytophaga-flavobacter-bacteroidetes
CFU	Colony forming unit
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
EPA	Environmental Protection Agency
FCR	Feed conversion ratio
FISH	Fluorescence in situ hybridization
g	Gram
h, hrs	Hours
ha	Hectare
kg	Kilogram
L	Liter
mg	Milligram
mL	Milliliter
m^2	Square meter
m	Meter
M	Molar
mV	Millivolt
MPN	Most-probable number
NCBI	National Center for Biotechnology Information
nm	Nanometer
NOB	Nitrite oxidizing bacteria
NTU	Nephelometric turbidity units
ORP	Oxidation reduction potential

OUT	Operational taxonomic unit
PCR	Polymerase chain reaction
PSU	Practical salinity units
rpm	Revolutions per minute
rDNA	Ribosomal DNA
RDP II	Ribsomal Database Project II
rRNA	Ribosomal RNA
SD	Standard deviation
SEM	Scanning electron microscope
SRB	Sulphate reducing bacteria
t	Metric ton
TAN	Total ammonia
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
µg	Microgram
µL	Microliter
V	Volt
γ	Gamma
β	Beta
α	Alpha
δ	Delta

CHAPTER I

INTRODUCTION

1.1 Background

Aquaculture in earthen pond is typically used worldwide. Biological processes such as photosynthesis and heterotrophic decomposition usually play an important role in pond stability. Hence, the success of aquaculture in earthen pond is depended on waste management by either natural or man-made processes.

It is known that nitrogen is one of the most important wastes found in aquaculture pond. Sources of nitrogen input to aquaculture pond are uneaten feed and organic waste excreted from animals. In shrimp pond, the major input of nitrogen waste comes from protein in artificial feed pellet. Because only 20-30 % of nitrogen in feed is converted into shrimp biomass while the rest is excreted and accumulated in high organic content sediment at the pond bottom (Acosta-Nassar *et al*., 1994; Hargreaves, 1998; Burford and Williams, 2001). At the end of each shrimp culture crop in Thailand, farmer removed high organic sediment from shrimp pond using pressurized water hose and drained the sediment out of the farm. This has been recently prohibited by the environmental regulation by the Department of Fisheries. With the Good Aquaculture Practice approach, every farm must retain waste within the farm by mean of recirculating water among aquaculture pond and reservoir. Apart of sediment removal every time after cultivation crop, the alternative treatment of organic waste by aerobic and anaerobic digestion through the consequential ammonification, nitrification and denitrification processes was proposed with this study.

Decomposition of organic waste results in ammonia-nitrogen release. High ammonia concentration in shrimp pond is usually found after phytoplankton bloom and follow up by nitrite accumulation. Thus, the sediment–water interface in earthen ponds is a sink and a source of various substances that are potentially toxic for cultured species (Avnimelech and Ritvo, 2003; Kassila, 2003). Release of ammonia from sediment is mainly from ammonification process after the decomposition of organic matters including phytoplankton and uneaten feed. In nature, toxic inorganic

nitrogen compounds *i.e.* ammonia-nitrogen and nitrite-nitrogen are converted to non-toxic nitrate-nitrogen by nitrification process. High concentration of nitrate in aquaculture pond can result in eutrophication, however, this is rarely occurred since nitrate is eliminated from the pond by denitrification process in sediment (Boyd, 1995; Burford and Lorenzen, 2004).

Nitrification is the biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of nitrite into nitrate. This process plays a central role in the nitrogen cycle and is often a critical first step in nitrogen removal of aquatic environments including the base of the euphotic zone, suboxic water columns, estuarine and coastal sediments (Dorigo *et al*., 2005; Francis *et al*., 2005; Cafferey *et al*., 2007). Ammonia oxidation plays by the ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (de Boer, *et al*., 2001). For nitrite oxidation, it is carried out by nitrite oxidizing bacteria (NOB) including *Nitrobacter*, *Nitrococcus*, *Nitrospina* and *Nitrospira*. Of these, NOB is belonging to subclass Alpha-, Gamma- and Delta-proteobacteria and class Nitrospirales (Hovanec *et al*, 1998; Jie *et al*, 2008). As several publications reports that the regulation of nitrification potential was correlated with ammonia concentration (Campos *et al*., 2002), the C:N ratio (both total organic:total nitrogen and total inorganic:total nitrogen) (Jie *et al*., 2008; Guisasola *et al*., 2006; Wett and Rauch, 2003), dissolved oxygen (Kim *et a*l., 2005; Jianlong and Ning, 2004), pH (Tarre and Green, 2004;) and temperature (Zhu and Chen, 2002). Most of previous factors were studied in the waste water treatment however some of them were investigated in soil and also sediment (Henriksen, 1980; Bodelier *et al*., 1996). Fierer and Schimel (2002) showed that moisture in soil from annual grassland in Santa Ynez, California, USA had affect on the nitrifying activity and its community. Increased of pH in sediment from West Lake, China, result in release of ammonia to water column increased (Hu *et al*., 2003).

Denitrification is an anaerobic microbial respiration process with a stepwise reduction of nitrate or nitrite via nitric oxide to nitrous oxide or dinitrogen which is subsequently released into the atmosphere. Heterotrophic denitrifying bacteria are capable of using the chemically bound oxygen in nitrate as a terminal electron acceptor and also utilize organic carbon for an electron donor (Mateju *et al.*, 1992). To enhance denitrification process, various organic carbon compounds such as cellulose, molasses, hydrolyzed corn straw, acetic acid, propanol, butanol, glycol,

methane, glucose, ethanol and methanol have been used as the substrates for denitrifying bacteria (Sobieszuk and Szewczyk, 2006; Shrimali and Singh, 2001; Ferguson, 1994; Mateju *et al*., 1992). However, the efficiency of denitrification depends on the type of carbon source and also the C:N ratio (Hamazah and Ghararah, 1996; Menasveta *et al*., 2001).

Since the proper management of nitrogenous waste strongly affects on water quality in aquaculture pond, this study aims to examine the role of natural processes with emphasis on microbial activities at the pond bottom soil that influencing or enhancing nitrogenous waste conversion in both laboratory and outdoor conditions. The effect of environmental factors on microbial community and nitrogen conversion was also investigated.

1.2 Objectives

1.2.1 To investigate inorganic nitrogen conversion and bacterial diversity in pond bottom soil from shrimp pond under laboratory and outdoor artificial aquaculture pond conditions.

1.2.2 To study the effect of environmental condition *i.e.* salinity, dissolved oxygen, organic and inorganic carbon addition and soil drying on nitrogen conversion rate and bacterial diversity in pond bottom soil.

1.2.3 To evaluate the carrying capacity of aquaculture pond based on the natural nitrogen treatment in sediment.

1.3 Hypothesis

1.3.1 There is a relationship between nitrogen conversion process and bacterial diversity in pond bottom soil.

1.3.2 Changes of environment conditions could affect either nitrogen conversion rate and microbial diversity of the pond bottom soil.

1.3.3 Most of the nitrogen conversion processes are related to the microbial activities at the surface of pond bottom soil. Therefore, nitrogen conversion rate of the pond bottom can be used to evaluate the carrying capacity of aquaculture pond.

CHAPTER II

LITERATURE REVIEWS

2.1 Earthen ponds

Most soils are stratified into horizontal layers which called soil profiles. Figure 2.1 shows soil profile in aquaculture pond. The F and S (aerobic zone) horizons contain most of the living organisms and organic matter. In addition, these horizons are important for pond soil management because they exchange many substances with overlaying water influence water quality (Boyd, 1995; Munsiri *et al*., 1995).

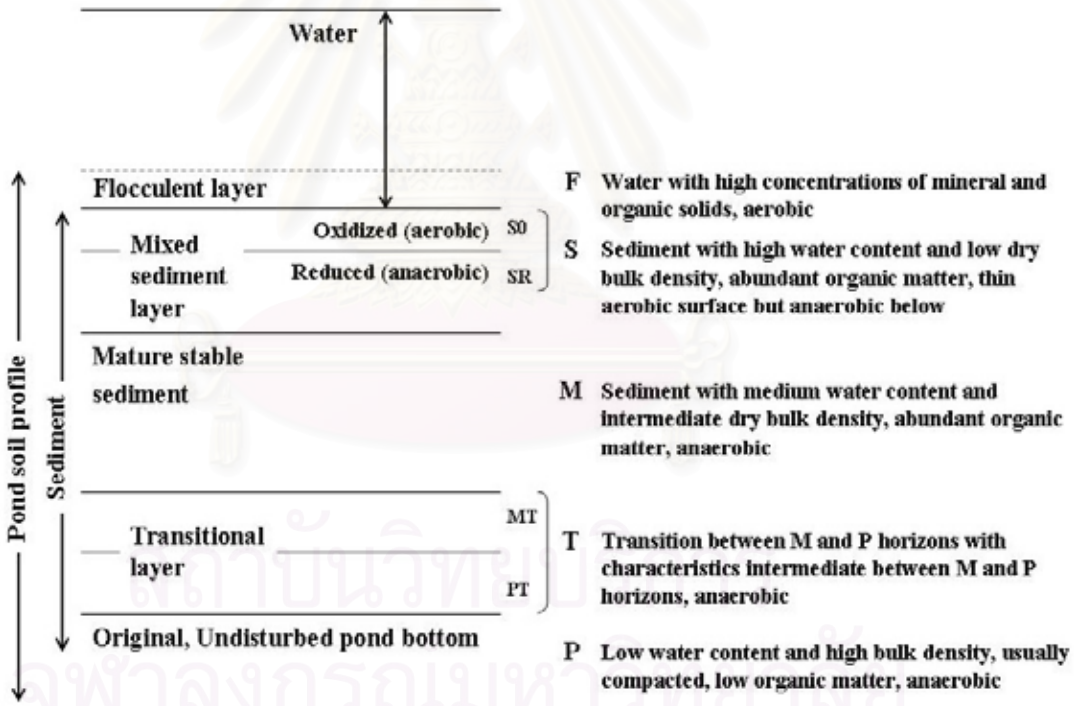


Figure 2.1 Pond bottom soil profile showing possible soil horizons (Munsiri *et al*., 1995).

Decomposition of organic matter by aerobic bacteria produces ammonia. In anaerobic condition, some bacteria can decompose organic matter by fermentation process. Anaerobic bacteria, on the other hand, are able to use oxygen from nitrate, nitrite and sulfate while they release nitrogen gas, ammonia and hydrogen sulfate (Prosser, 1986). Releasing of these toxic substances to water column is harmful for cultured organisms. Under normal practice of aquaculture, oxidized layer of the sediment surface should be maintained throughout the crop (Boyd *et al*., 2002). Most toxic substances are oxidized to non-toxic form by biological activity at the oxidized layer before passing into water column. This includes the oxidation of nitrite to nitrate, ferrous to ferric iron and hydrogen sulfide to sulfate.

2.2 Inorganic nitrogen compound in aquaculture pond and their affect on cultured organisms

The potential pollution in aquaculture pond water is depended on the level of fish and shrimp density. Water quality in aquaculture ponds, which is suitable for raising high density for the cultured animal, must be managed by the specific regime because high nutrients, organic matter and solid suspended, low dissolved oxygen and high biological oxygen demand (BOD) are evident in the intensive system. So the pollution removal potential in intensive pond must greater than in extensive and semi-intensive. Typical the water qualities in three systems are given in Table 2.1.

Table 2.1 Typical range for selected water quality variables in water column of aquaculture ponds and three levels of production (Egna and Boyd, 1997).

Water quality	**Level of aquaculture production**		
	Extensive (<1000 kg/ha)	**Semi-intensive (1000-5000 kg/ha)**	**Intensive (>5000 kg/ha)**
Chlorophyll-*a* (µg/L)	100-50	50-150	150-500
BOD (mg/L)	2-5	5-20	20-40
Volatile solid (mg/L)	5-10	10-20	20-50
Turbidity (NTU)	5-10	10-25	25-50
Nitrate-nitrogen (mg/L)	0.001-0.1	0.1-0.2	0.2-0.3
Total ammonia (mg/L)	0.1-0.5	0.5-2	2-5
Total Kjeldahl nitrogen (mg/L)	0.5-2	2-4	4-10
Total phosphorus (mg/L)	0.05-0.1	0.1-0.3	0.3-0.7
Settleable solids (mg/L)	0-0.05	0.05-0.1	0.1-0.5

Concerns are evoked about the possible effects of aquaculture wastewater both on productivity inside aquaculture ponds and on the ambient aquatic ecosystem. Nitrogenous compounds are considered major contaminants in aquaculture wastewater. There are three types of inorganic nitrogen compound as follows:

2.2.1 Ammonia

Ammonia exists in two forms: un-ionized (NH_3-N) and ionized NH_4^+. The sum of the two (NH_3+NH_4^+) is called total ammonia or simply ammonia (TAN). It is common in chemistry to express inorganic nitrogen compounds in terms of the nitrogen they contain *i.e.* NH_3, NH_4^+, NO_2^- and NO_3^-. This allows for easier computation of total ammonia-nitrogen and easy conversion between the various stages of nitrification (Timmons *et al*., 2002).

As NH_3 is one of the most toxic substances produced by intensive fish farms and has a high impact on aquatic communities. The chemical equilibrium between NH_3 and TAN is a function of salinity, temperature and, to a large extent, pH (Boyd, 1995). The release of dissolved nutrients by a fish farm leads to an increase in their concentration in the receiving water body. This increase has been called ‘‘hypernutrification’’ (Gowen and Bradbury, 1987). A large number of scientific investigations have been carried out on ammonia toxicity in fish farming and on water quality of rearing.

In confined areas such as pools, tanks, ponds, especially with inadequate water renewal, ammonia may reach concentrations strong enough to cause serious damage to the health and growth of the farmed animals, with sometimes lethal effects (Porrello *et al*., 2003). For fresh water, Colt and Tchobanollus (1978) found an inverse correlation between farmed animal growth and undissociated ammonia concentration. Alabaster *et al.* (1979) indicate a value of 0.025 mg/L of NH_3 as a safety limit for temperatures higher than 5°C. An investigation covering the entire life cycle of the rainbow trout (Thurston *et al*., 1984) revealed no effects on survival, growth or reproductive capacity at concentrations of 0.074 mg/L and set a value of 0.031 mg/L as histopathological value. Poxton and Allouse (1987) reported an appreciable increase in NH_3 levels as a function of feeding; this increase is also related to the method used for food distribution. Critical values of NH_3 for farmed

animals are much more difficult to ascertain in the presence of large variations in concentration. Exposure to high concentration peaks may actually be more detrimental than exposure to high but constant values owing to the animal's failure to adapt to the environment (Ruffier *et al*., 1981). Toxic effects of undissociated ammonia on aquatic organisms in the concentration range 0.09–3.35 mg-N/L have been reported by Handy and Poxton (1993). The literature data are refer to only a small number of fish species on which experiments have been carried out under different conditions (pH, temperature, dissolved oxygen), which makes them difficult to compare. One valid approach seems to be that proposed by Porrello *et al.* (2003) in which the non-ionized ammonia concentration range within which no apparent damage is caused to the farmed animals is set at 0–0.01 mg-N/L for freshwater species.

2.2.2 Nitrite

Nitrite is a naturally occurring intermediate product in two bacteria-mediated process involving transformations of nitrogen in water and soils. Nitrite accumulates in aquaculture systems and can be toxic to aquatic animals. Acute toxicity of nitrite has been investigated in a number of fishes, crustaceans, molluscs and echinoderms.

Nitrite enters a fish culture system after feed is digested by fish and the excess nitrogen is converted into ammonia, which is then excreted as waste into the water. Total ammonia nitrogen (TAN) is then converted to nitrite which, under normal conditions, is quickly converted to non-toxic nitrate by naturally occurring bacteria. Nitrite problems are typically more likely in closed, intensive culture systems due to insufficient, inefficient, or malfunctioning filtration systems. High nitrite concentrations in ponds occur more frequently in the fall and spring when temperatures are fluctuating, resulting in the breakdown of the nitrogen cycle due to decreased plankton and/or bacterial activity (Moriarty, 1997). A reduction in plankton activity in ponds can result in less ammonia assimilated by the algae, thus increasing the load on the nitrifying bacteria. If nitrite levels exceed that which resident bacteria can rapidly convert to nitrate, a buildup of nitrite occurs, and brown blood disease is a risk.

2.2.3 Nitrate

Nitrate is identified as one of the hazardous contaminants in potable water that may reduce to nitrosamines in the stomach which is suspected to cause gastric cancer (Glass and Silverstein, 1999). In addition, nitrate exposes infants and pregnant women to danger due to the potential reduction of nitrate to nitrite inside the stomach of foetus after digesting nitrate by the mother. The nitrite reacts with the hemoglobin in blood and converts the hemoglobin into methaemoglobin, which does not carry oxygen to cell tissues. This phenomenon results in a bluish color of infant's skin so called methaemoglobinemia or the blue baby syndrome (Shrimali and Singh, 2001). Most of the researchers reported that contamination of groundwater by nitrate is primarily attributed to non-point agricultural sources. Nitrate originated from agriculture is increasingly growing all over the world due to the extreme use of fertilizers. Nitrate salts reach the groundwater as they percolate through the soil. Some other sources of nitrate in ground and surface water are from uncontrolled land discharges of treated or raw wastewater from domestic and industrial wastes, landfills and animal wastes particularly from animal farms (Terada *et al.*, 2003).

Cultivation of river and marine organisms in their natural or synthetic habitat is always along with the production of ammonia (referring to NH_3 or NH^+_4) which is considered as a serious problem. Ammonia produces a bad flavor and taste as well as harmful effects on aquatic animals. EPA proposed that ammonia concentrations in fish culture tanks be maintained at levels lower than 0.02 mg/L as the criterion for non-ionic ammonia (EPA, 1976). Therefore, indispensable need for ammonia removal complied with applying different pathways such as nitrification, sludge removal by sedimentation or mechanical filtration, and water exchange (van Rijn, 1996). Nitrification is reported as one of the most common and effective methods applied by researchers (Zhu and Chen, 1999) in both freshwater and seawater. In this method ammonia is oxidized into nitrite and finally nitrate as the end-product. Reported maximum nitrate levels differ among various systems and values of 400–500 mg-N/L were reported (Otte and Rosenthal, 1979). Contrary to ammonia and nitrite, nitrate is relatively non-toxic to aquatic organisms. However, it should not be left to accumulate, because eventually leads to some undesirable results such as phytoplankton blooms, inhibition of nitrification and toxicity problems at

certain concentrations of nitrate (van Rijn *et al*., 2006). As a result, daily replacement of a fraction of the system volume (5–10%) with new water may prevent accumulation of nitrate, though this solution does not sound environmentally. Increased efforts are now directed towards nitrate control in aquaculture systems, not only concerning toxic effect on fish, but also for complying to environmental regulations associated with permissible nitrate level in effluent discharge which is as low as 11.3 mg-N/L (Otte and Rosenthal, 1979).

2.3 The important processes associated with nitrogen dynamic in aquaculture ponds

Nitrogen plays a key role in the dynamics of aquaculture systems. Apart of nitrogen that acts as a major nutrient for plants, accumulation of inorganic nitrogen compounds especially ammonia and nitrite is toxic to cultured organisms. Main processes associated with nitrogen cycle in pond are:

2.3.1 Mineralization

Organic compounds are usually decomposed to inorganic compounds by heterotrophic bacteria. In aquaculture pond, decomposition of organic matter encounters in the flocculent sediment layer, approximately 2 cm above the sediment surface (Visscher and Duerr, 1991). Increase of organic matter accumulation results in the high oxygen demand and oxygen depletion which enhance an anaerobic decomposition. This is probably happen at a thin aerobic layer on top of the sediment (Brown *et al*., 1987).

2.3.2 Nitrification

Nitrification is a sequential biological oxidation process involved with two groups of bacteria. The first step of nitrification is ammonia oxidation by ammonia oxidizing bacteria (AOB) which oxidizes ammonia to nitrite via hydroxylamine (NH_2OH). The following step, nitrite oxidation, is the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB). Autotrophic nitrification is

confined to a relatively narrow range of bacteria. All known terrestrial AOB belong to a monophyletic group within the β-subclass of the Proteobacteria, and the currently accepted classification recognizes only two genera within this group, *Nitrosospira* and *Nitrosomonas* (Head *et al*., 1993). A single genus within the γ-subclass Proteobacteria, *Nitrosococcus*, also exhibits the property of ammonia oxidation, but its apparent restriction to marine environments. Representatives of the NOB occur in a number of phylogenetic groupings. Autotrophic NOB are *Nitrobacter* (α-subclass Proteobacteria), *Nitrococcus* (γ-subclass Proteobacteria), *Nitrospina* (δ-subclass Proteobacteria), and *Nitrospira* (separate phylum of the Bacteria). Of these, only *Nitrobacter* has been detected in soils, and the natural distribution of other NOB genera remains to be fully investigated. Ammonium oxidation is often thought to be the rate-limiting step in autotrophic nitrification.

A wide phylogenetic range of bacteria and fungi possess the potential for heterotrophic nitrification, and the range of transformations includes the oxidation of both organic and inorganic nitrogen compounds. In contrast to AOB, oxidation of ammonium by heterotrophs is not linked to cellular growth (Fenchel *et al*., 1998). Two biochemical pathways have been proposed for the oxidation of ammonium by heterotrophs. The first pathway is known from heterotrophic nitrifying bacteria such as *Paracoccus denitrificans*, *Thiosphaera pantotropha*, *Pseudomonas putida* and *Alcaligenes faecalis* (Jianlong and Ning, 2003; Rittman, 2006). These bacteria possess ammonia and hydroxylamine oxidizing enzymes that have strong similarities with those of autotrophic nitrifiers. Some of the heterotrophic nitrifying bacteria, such as *Thiosphaera pantotropha*, combine their nitrifying activity with aerobic denitrification (Bodelier *et al*., 1996). This combined nitrification–denitrification process appears to be used for dissipating reducing equivalents under conditions where the oxygen respiration capacity is limited. By doing so, such organisms can maintain a high growth rate, which can be advantageous when energy substrates are in excess (Barak and van Rijin, 2000). For heterotrophic nitrifying bacteria that are not capable of aerobic denitrification, the production of nitrogen oxides may provide a mechanism to inhibit the growth of competing microorganisms.

The availability of ammonia depends on the C:N ratio of the dissolved organic matter being mineralized. A high C:N ratio results not only in a small production of ammonia but also in the large amount of carbon that consumes oxygen.

Nitrification efficiency is highest when oxygen penetration into the sediment is greatest. Under these conditions, there is least diffusion loss of ammonia which comes from the lower sediment layers to the oxygenated zone. The location of the dissolved organic matter source close to the sediment surface results in disproportional high losses of both dissolved organic nitrogen and ammonia. This results in decrease in nitrification rate (Fenchel *et al.*, 1998). Nitrification can be found in water column and at sediment surface. Nitrification rate in water column of Narragansett Bay and Chesapeake Bay was 0.16 and 0.45 mg-N/L/day, respectively (Berounsky and Nixon, 1993; Horrigan *et al*., 1990). Examples of nitrification rate in sediment, on the other hand, are shown in Table 2. In water column, nitrification is probably inhibited by light. Insufficient surface for bacterial attachment is also the limitation of nitrification rate. It is known that nitrification is essential for nitrogen treatment in intensive aquaculture. The attempt to increase nitrification rate is therefore investigated. Major site for nitrification and mineralization in water column is commonly found incorporate with suspended particle (Wasailesky *et al*., 2006). High rate of nitrification is also found at the sediment-water interface. Ammonia concentration, dissolved oxygen concentration, temperature, pH and number of nitrifying bacteria are among the most important factors that affects nitrification process (Gross *et al*., 2000; Bufford and Longmore, 2001; Caffrey *et al*., 2003).

2.3.3 Denitrification

Denitrification is a nitrogen reduction process in which at least 14 bacteria genera can reduce nitrate or nitrite to nitrogen gas in the absence of O_2. (Focht and Verstraete, 1977). It is performed mainly by facultative anaerobic bacteria that utilize organic (heterotrophic denitrification) or inorganic (autotrophic denitrification) compounds as electron sources to reduce nitrate (Prosser, 1986). In aquaculture ponds, ammonia and nitrite is typically maintained at low levels by daily water exchange. Accumulation of nitrate can be found if high nitrification was occurred. Denitrification is usually found in an anoxic layer below sediment surface. Thick anoxic layer is not only inducing high denitrification rate but sulfate reduction is also occur. This leads to the generation of toxic sulfide (H_2S). Denitrification rates as reported in literatures are shown in Table 2.2.

Table 2.2 Nitrification and denitrification rate estimates in marine and freshwater sediment.

Process	Source of sediment	Rate of reaction (mg-N/m^2/day)	Reference
Nitrification	Freshwater fish pond	0.4-0.9	Acosta-Nassar *et al*., 1994
	Polyculture fish pond, Thailand	1-35	Riise and Roos, 1997
	Hiroshima bay, Japan	0-7.16	Kim *et al*., 1997
	Seneca river, USA	32	Pauer and Auer, 2000
Denitrification	Freshwater, fish pond	0.1-7.1	Acosta-Nassar *et al*., 1994
	Freshwater, polyculture fish pond, Thailand	57	Riise and Roos, 1997
	Hiroshima bay, Japan	0-19	Kim *et al*., 1997
	Enriched marine sediment mesocosm	101-296	Seitzinger *et al*., 1984
	enriched, bioturbated marine sediment	420-490	Binnerup *et al*., 1992

2.3.4 Phytoplankton uptake

Phytoplankton consumes ammonia as a nutrient for growth. Ammonia uptake by phytoplankton is an important ammonia removal process from the water. Rate of nutrient uptake by plankton is limited by proportionally of nutrients (C:N:P) and light intensity (Moriarty, 1997). Ammonia is an admired substrate for phytoplankton. If it has been depleted to lower than 0.03 mg-N/L, nitrate will be assimilated (van Rijin *et al*., 1986). In sediment exposed to light, benthic microalgae can also increase the efficiency of ammonia removal.

Microalgae from water bodies that supply water are founding the early stages in shrimp farm ponds. Phytoplankton composition and abundance in supply water is modified in shrimp ponds. In some culturing systems, where salinity

decreases because of the mixing with fresh water from rivers, there are ponds where diatoms, cyanobacteria, chlorophytes and dinoflagellates dominate, depending on several environmental factors (*e.g.* light, salinity, temperature and nutrient levels). The occurrence of some species can be temporal or can last longer. Sometimes there are blooms of short periods, but a very high abundance of one or a few species that can alter shrimp growth due to oxygen depletion at nights (depending on density, dominant species and bloom duration). Within the first weeks of the culture (when shrimp change from postlarvae to juvenile), specimens feed on microalgae and planktonic copepods, detritus and mollusk larvae. In semi-intensive systems, food supply starts 2 months after the stocking, depending on the quality of supplied water since post-larvae feed on the naturally occurring food (Fast, 1992). The main contribution of phytoplankton to the sub-adult and adult stages of cultured shrimp is through the trophic chain: shrimp can feed on macrofauna such as small bivalves and gasteropods; on meiofauna such as polychaetes, amphipods and harpacticoid copepods; and on meiobenthos such as bacteria and detritus. Shrimp also consumes phytoplankton when it is adhered to detritus. Protein, lipid and carbohydrate content in phytoplankton vary among species and because of environmental factors. With respect to amino acid content, almost all microalgae have a similar composition (Brown *et al*., 1997). According to Alonso-Rodrıguez and Paez-Osuna (2003), diatoms have higher growth rate than cyanobacteria. Most shrimp farm managers prefer a high ratio of diatoms in a phytoplankton community; this is accomplished by repeated small amounts of fertilizers that results in a N:P ratio of 20:1 (Boyd and Daniels, 1993).

Boyd (1995) has established that diatoms are the dominant phytoplankton group in ponds with brackish waters, while cyanobacteria dominate in ponds with waters of lower salinities in temperate waters. Nevertheless, in shrimp farms from NW Mexico, cyanobacteria was the dominant group, followed by dinoflagellates and diatoms; a similar situation has been reported in other subtropical regions of the world. Variations of phytoplankton in a semi-intensive farm from NW Mexico showed that to salinities 16–20 PSU, dominant species were cyanobacteria (*Synechocystis diplococcus* and *Oscillatoria limnetica*) and a dinoflagellate (*Prorocentrum minimum*). In another study, in two shrimp farms located in southern Sinaloa, N:P ratios were 6.8 for a semi-intensive system and 3.6 for an intensive

system. In both farms, cyanobacteria were dominant, with a highest abundance of $3.5x10^6$ cells/L; other important groups were diatoms, dinoflagellates and phytoflagellates. Euglenophytes were also registered during the culture cycle.

In semi-intensive pond waters where phytoplankton barely exceeded $1x10^6$ cells/L, it was mainly composed of nanoplankton community. It has been observed that after the first half of the culture cycle, density in intensive and semi-intensive systems was higher than $1x10^6$ cells/L. For the studied ponds in four shrimp farms in NW Mexico, cyanobacteria was the dominant group numerically, and with the exception of an intensive farm (89%), abundance was always >98%. The above results agree with the observations of Sevrin-Reyssac and Pletikosic (1990) for a higher abundance of cyanobacteria during summer.

2.4 Transformation of nitrogen compounds in aquaculture ponds

2.4.1 Earthen pond with water exchange

In artificial feed pellet, sources of protein such as fish meal and soybean meal are among the major source of nitrogen input in the culture system. Several reports, as shown in Table 2.1, reveled that only 18-42% of nitrogen in feed could be converted into cultured organism biomass while the rest is released into the environment. Large amount of nitrogen compounds are finally accumulated in high organic content sediment at the pond bottom. Following decomposition, release of inorganic nitrogen compounds is an important factor that limiting feeding rate of cultured species (Burford and Williams, 2001). Additionally, excretion of ammonia by cultured organism and remineralization of nitrogen in soil organic matter also influence nitrogen dynamic in the pond as they may be transformed via numerous of pathways. Ammonia can be absorbed by phytoplankton, converted via nitrite to nitrate by nitrification process and volatilized as gaseous ammonia. Nitrate is assimilated by phytoplankton and it can be discharged during water exchanges (Figure 2). However, nitrate reduction by denitrification process is simultaneously found in aquaculture soil (Acosta-Nassar *et al*., 1994; Riise and Roos, 1997; Burford and

Longmore, 2001). As a result of this process, nitrate is reduced to nitrogen gas and released to the atmosphere.

A conceptual model of nitrogen dynamics in shrimp ponds (Lorenzen *et al*., 1997) was extended to incorporate a sludge nitrogen pool and the remineralization process (Figure 2.1). For the purposes of the model, nitrogen input was assumed to be exclusively formulated feed since previous studies have shown that only a small proportion of the nitrogen input was in the form of water and stock (5–10%) (Briggs and Funge-Smith, 1994; Jackson *et al.*, 2003). In the model, nitrogenous waste is generated from shrimp feeding on formulated feed. Nitrogen can enter the water column as TAN (total ammonia nitrogen) from shrimp excretion or remineralization of wasted feed and as dissolved organic nitrogen. TAN may be transformed via a number of pathways: assimilated by phytoplankton, volatilized as gaseous ammonia, converted to nitrate via nitrite via nitrification processes or discharged during water exchanges. Nitrate may be assimilated by phytoplankton or discharged during water exchanges. A previous study by Burford and Longmore (2001) measured denitrification directly and showed that the sediment and particularly the sludge were highly anoxic, and nitrate concentrations were low, thereby preventing denitrification.

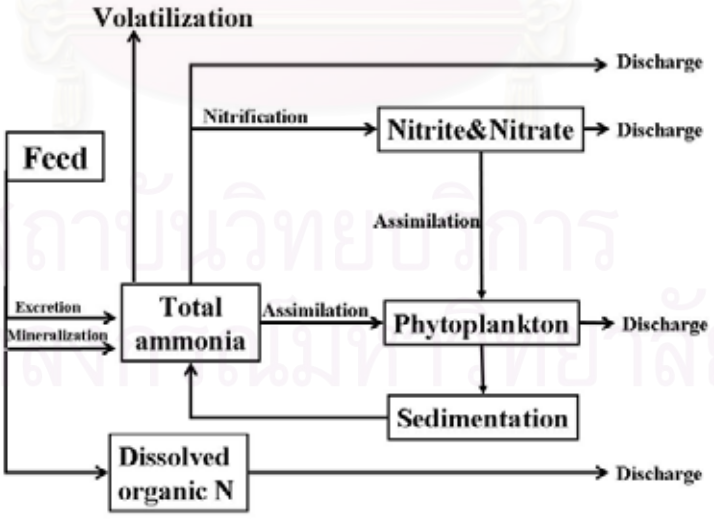


Figure 2.2 Model of nitrogen input, transformation and removal in earthen ponds (Burford and Lorenzen, 2004).

The two significant components of the pond environment are the pond water and sediments which interact continuously to influence the culture environment. Pond sediments can be further divided into the pond soil component the pond bottom and walls and the accumulated sediment component the sludge that accumulates on the pond bottom during culture Briggs and Funge-Smith, (1994). Pond management activities are a third external factor which influence the culture environment. Management activities include feeding, use of aerators, water exchange and liming. The original method of intensive shrimp culture in Thailand involved relatively high stocking densities 50–100 m, high production 6–12 t/ha/crop, high feeding rates FCR 1.8–2.0 and high rates of water exchange up to 5–10% per day towards harvest. Water quality management was achieved by a combination of flushing the pond with clean seawater and management of the phytoplankton bloom by assessment of pond colour. Water exchanges were frequent, especially in the latter half of production. Accumulated sediment was known to be undesirable and was removed between cycles. Experience has taught farmers that inadequate sediment removal would cause water quality problems early in the subsequent crop. Due to the high flushing rates and naturally high levels in seawater, the alkalinity of the pond water was not considered important and no attempt was made to control pH.

2.4.2 Lining pond

One method by which many problems of earthen ponds especially low water exchange systems can be avoided is the use of pond lining. Full pond liners bitumen impregnated geotextile have great potential in separating the pond bottom from the water column at the Tinsulanonda Songkhla Fisheries College (Boyd, 1995). This eliminates soil erosion and reduces the accumulation of sediment in the centre of the pond, resulting in a larger clean feeding area for the shrimp and quick but efficient pond cleaning typically 1–2 days can be reduced to 3 h with hose for lined pond. Very little waste is left in the pond after harvest and the dry out time required for earth ponds is not necessary. The characteristic of the small volume of accumulated sediment found in lined ponds is completely different for that of earthen ponds. Accumulated sediment in lined ponds is not consolidated 83% water and 62% water in earth ponds, remains extremely liquid and can be easily pumped out of the pond. The lack of soil in the accumulated sediment of lined ponds causes it to have a higher

organic content 36% than in accumulated sediments of an earth pond (Boyd, 1995). This high organic content is also reflected in the higher levels of leachable nutrients in the accumulated waste of lined ponds. The higher levels of labile ammonia and dissolved reactive phosphorus in the lined pond waste have implications for sediment management since these nutrients can act as fertilizers and, if in excess, stimulate overblooming in the pond. On the other hand, removal of these nutrients can destabilize the phytoplankton bloom resulting in high water transparency. The former situation is becoming usual at the Fishery College. The high organic content of accumulated sediment from lined shrimp ponds can also be used as fertilizer after desalting (Briggs and Funge-Smith, 1994).

Despite the promise that lined and partially lined ponds can improve water quality, production from these systems has not been fully convincing, possibly because new management techniques are required. One reason for the observed cannibalism and occasional high FCR in lined ponds is the tendency for feeds to be rapidly carried to the centre of the pond with water circulation. The contribution of the pond soil and detritus feeding to shrimp nutrition and growth is uncertain in intensive ponds, lined ponds might limit the availability of some nutrients to the shrimps. By forming a barrier between the pond soil and the water, anaerobic conditions develop beneath the liner.

Adequate drainage must be provided to allow exit of seepage water. Gas formation below the liner can cause it to float so weighting is also required typically concrete strips or fencing posts. If pond soil is potential acid sulphate, the conditions below the liner can become extremely toxic. In such situations, sand can be applied between the soil and liner to facilitate drainage. Care must be taken to avoid seepage from adjacent unlined ponds and canals. The principal drawback of liners in shrimp culture ponds is their high cost and relatively short service life. More expensive liners last longer, but care must be taken to avoid deterioration from exposure to sunlight if there is extended time between crops. Ponds should be full of water even if there is no stock. Disposal of old lining materials will become a problem if wide scale adoption occurs, since burning them appears to be the most likely method.

2.5 Assessment of microorganism diversity in aquaculture pond

2.5.1 Principle of denaturing gradient gel electrophoresis (DGGE) separation

Amplification of DNA extracted from mixed microbial communities with primers specific for 16S rRNA gene fragments of bacteria result in mixtures of PCR products. Because these products all have the same size, they cannot be separated from each other by agarose gel electrophoresis. However, sequence variations between different bacterial rDNA bring about different melting properties of these DNA molecules, and separation can be achieved in polyacrylamide gels containing a gradient of DNA denaturants, such as a mixture of urea and formamide. PCR products enter the gel as double-stranded molecules; as they proceed through the gel, the denaturing conditions gradually become stronger (Kowalchuk *et al.*, 1999). PCR products with different sequences therefore start melting at different positions (*i.e.* at different denaturant concentrations) in the gel. Melting proceeds in so called melting domains. Once a domain with the lowest melting temperature reaches its melting temperature at a particular position in the denaturant gradient, a transition from a double-stranded to a partially melted molecule occurs. The protruding single strands practically cause a halt of the molecule at that position. To prevent the complete dissociation of the two DNA strands, a 40-nucleotide GC-rich sequence (GC-clamp) is attached at the 5'-end of one of the PCR primers.

2.5.2 DGGE to study spatial and temporal variability of bacterial populations

The distribution of microbial populations in the marine water column depends on numerous factors and variables. Especially in stratified systems exhibiting strong physicochemical gradients, DGGE fingerprinting can reveal a concomitant stratification of resident microbial assemblages. Most-probable number (MPN) counts of SRB were done in parallel and showed a similar trend for the distribution of sulphate reducing bacteria (SRB). Interestingly, DGGE patterns of PCR products

obtained from cDNA after reverse transcription of RNA, representing the active populations, were different from those obtained after amplification of genomic DNA. Despite the agreement between MPN and DGGE, the hybridization of DGGE patterns with oligonucleotide probes and sequencing analysis of DGGE bands revealed that the SRB enriched in the MPN-tubes had a different phylogenetic affiliation from the SRB detected in the natural samples (Wleringa *et al.,* 2000). The finding that SRB obtained from the MPN cultures belonged to the genera *Desulfiovibrio, Desulfobulbus, and Desulfobacter*, but those in the DGGE patterns of natural samples represented an independent lineage of the Proteobacteria, verified the potential disagreement between culture-dependent and molecular methods due to selection of culturable types of SRB.

The potential of PCR-DGGE for the analysis of large sets of samples was recognized by Ferrari and Hollibaugh (1999). They processed 100 samples from different stations in the Arctic Ocean to analyse the spatial variation in the diversity of bacterioplankton assemblages. DGGE fingerprints of the samples were subjected to image analysis and the spatial variation of the bacterioplankton assemblage was inferred by regression analysis of the similarity of densitometric curves derived from the DGGE patterns. The resulting dendrogram separated all DGGE patterns into five major clusters with minimally 80% similarity. While clustering of some samples corresponded to samples taken in a specific region of the Arctic Ocean, there was no correlation of geography and clustering of other samples. The authors noted that clustering of the majority of samples rather seemed to reflect different phases of the cruise and might therefore be confounded with temporal variation over the 44 days period of the cruise (Ferrari and Hollibaugh, 1999). The bacterioplankton assemblages of two estuaries in California, San Francisco Bay and Tomales Bay, differing markedly in a number of physical and biological factors, had been shown to differ in metabolic properties.

The diversity of microorganism in aquatic environmental has now generally been estimated on the basis of phenotypic characters. In fact, knowledge of microorganisms in nature is very limit due to their small size and the absent of phenotypic characters. Traditionally, biodiversity is based upon the species as a unit. Species diversity consists of two components, species richness and species evenness (distribution). For prokaryotic organisms the species concept is obscure. This problem

has been circumvented by replacing traditional identification and classification with numerical taxonomy. With this method the distances between isolates are calculated and they are then clustered into biotypes. The biotype is an operational taxonomic unit (OTU) which can be used instead of species to characterize and compare populations and communities. One gram of soil or sediment may contain more than 1010 bacteria as counted in fluorescence microscope after staining with a fluorescent dye (Pace, 1997; Torsvik *et al*., 2002). A serious problem in microbial ecology is that the relative proportion of bacteria growing on agar plates (CFU) varies from 0.1 to 1% in pristine forest soils to 10% in environments like arable soil. This implies that investigations based on bacterial isolates may include only a minor part of the total bacterial diversity. Since the last two decades, molecular identification techniques that do not require cultivation have been used instead of the classical technique. Molecular identification has been widely applied for many aspects especially with medical and environmental microbiology.

One of the popular techniques for studying microbial diversity is the denaturing gradient gel electrophoresis (DGGE) of PCR-amplified genes. This technique can be used to evaluate the diversity of complex microbial systems (Muyzer *et al*., 1993). With DGGE, separation of DNA fragments with equal length is based on sequence-dependent melting behavior in a polyacrylamide gel containing a gradient of denaturant concentration. Separation of double stands DNA depends on the hydrogen bonds formed between complementary bases pairs. Complete DNA separation is prevented by the attachment of a high melting domain; know as GC clamp, which is added to one primer set (Dorigo *et al*., 2005). DGGE is commonly used to separate mixed PCR products after the amplification of 16S ribosomal DNA (rDNA) fragments (Kowalchuk *et al*., 1999). DGGE technique has been applied to investigate the diversity of organisms in many diverse habitats (Muyzer *et al*., 1993; Kowalchuk *et al*., 1999).

2.5.3 DGGE to monitor population shifts after environmental perturbation

As pointed out above, PCR-DGGE analyses can be performed with DNA as well as with RNA. While DNA-derived PCR amplified 16S rRNA gene

fragments are related to the presence of different bacterial populations, analyses of rRNA-derived PCR products can give an indication of which bacterial populations contribute to the RNA pool. As the cellular concentration of ribosomal RNA is related to the activity of cells it helps in surveying changes in the activity of bacterial populations. Potential between DNA and RNA-derived was evaluated. Similarly, analysis of the genetic diversity and expression of functional genes can be performed using either DNA or mRNA. Here, PCR-DGGE analysis of DNA-derived PCR products show the genetic diversity (presence) of certain functional genes, while PCR products obtained after DNase digest and reverse transcription of mRNA show the diversity of expressed genes (Wawer *et al*., 1997). Rossello-Mora *et al*. (1999) investigated the response of the microbial community of marine sediments to amendment with cyanobacterial biomass under anaerobic conditions. Fluorescence in situ hybridization (FISH), DGGE of PCR products obtained from DNA as well as from cDNA after reverse transcription of RNA and sequencing of 16S rDNA PCR products were used to assess changes in the microbial community composition. Concomitant changes in the activity of the community were followed by measurements of carbon mineralization, sulphate reduction, and ammonium production rates. Addition of cyanobacterial biomass resulted in marked changes in the composition. Dominant bands from RNA derived banding patterns were affiliated with members of the cytophaga-flavobacter-bacteroidetes (CFB). FISH with probes specific for these CFB populations showed that, although sulphate reduction was the main mineralization process, members of the CFB, but not SRB showed the highest increase in abundance as detected by FISH. The authors concluded that these CFB played an important role in the anaerobic decomposition of complex organic matter and suggested that CFB might be responsible for hydrolysis of macromolecules and fermentation. Mesocosm experiments were performed by Lebaron *et al*. (2001) and Schiller *et al*. (2001) to study changes in the activity and diversity of bacterial assemblages from the Mediterranean Sea after addition of nutrients. Fluctuations in activity were recorded in parallel to variation in community composition, which was assessed by PCR-DGGE. Different phases were observed during the incubation corresponding to an initial increase of bacterial numbers, followed by an increase of heterotrophic protozoa cropping the bacterial production and a new increase of bacterial production after the peak in grazing activity (growth, grazing and post grazing phase, respectively). These phases were reflected by concomitant changes in

DGGE-fingerprints of the bacterial assemblage. Both, nutrient addition as well as grazing of protozoa seemed to effect changes in the bacterial genetic diversity. Multidimensional scaling analysis of DGGE patterns showed that differences in the development of the bacterial communities.

2.5.4 Eukaryotic microbial communities

Although small eukaryotes such as protozoa can be identified much easier by microscopy than bacteria because of their discriminative morphological features, their identification is time consuming and can often be done by experts only. Identification of eukaryotic microbes by molecular methods can be achieved with primers developed by van Hannen *et al*. (1998), which amplify a 210 bp 18S rRNA gene fragment that can be separated by DGGE. Due to the limited size of the fragment, sequencing of gel bands may make identification possible at the phylum level only (van Hannen *et al*., 1998). However, the authors demonstrate that, using specific oligonucleotide probes for hybridization analysis of DGGE gels, identification at the species level is possible. Van Hannen *et al*. (1998) used the eukaryote specific PCR-DGGE assay to compare the diversity of five Dutch lakes of a lagoon system. Analysis of DGGE fingerprints and environmental variables of these lakes by UPGMA resulted in similar clustering of lakes and the respective genetic fingerprints of their eukaryotic diversity.

CHAPTER III

GENERAL MATERIALS AND METHODS

3.1 Experimental framework

Experiments in this study were divided into two parts which presented in chapters 4 and 5 (Figure 3.1). Chapter 4 investigated the effect of environmental factors (*i.e.* salinity, DO, sodium bicarbonate, methanol, and illumination) on nitrogen conversion processes in sediment chambers under laboratory condition. The following chapter, Chapter 5, was an attempt to illustrate the effect of soil desiccation on nitrogen conversion and microbial diversity in the pond bottom soil under both laboratory and simulated outdoor pond. Materials and methods revealed in this chapter are general analytical techniques involved in all experiments. Detail of experimental designs and specific methods for each experiment are included in the next two chapters.

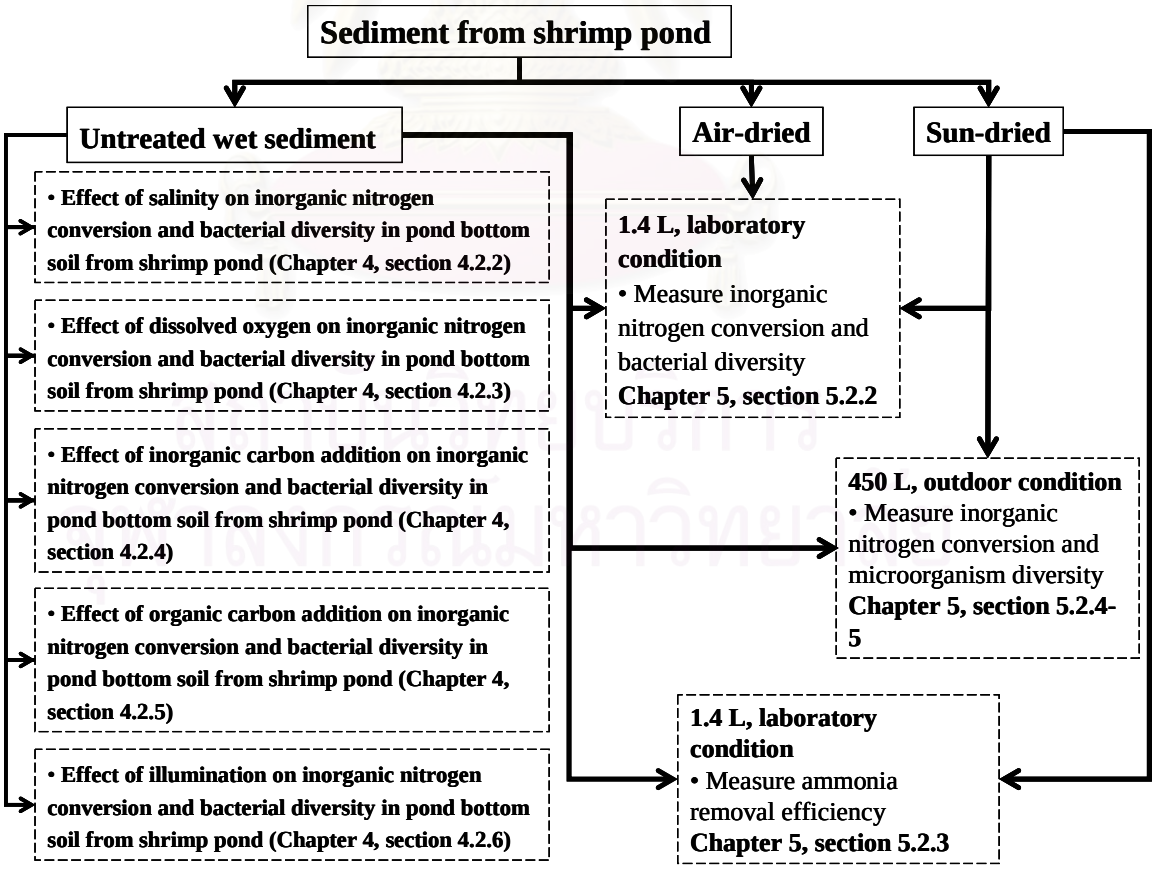


Figure 3.1 Framework of the experiments involved in this study.

3.2 Collection of sediment samples

Sediment samples in this study were collected from shrimp pond in Pathum Thani province, Thailand. This earthen pond was used for culturing Pacific White shrimp (*Litopenaeus vannamei*) under low salinity (3-6 PSU) with low water exchange rate. Surface layer of the sediment, approximate 5 cm in depth, was collected by grab sampler. Sediment samples were immediately transferred under cold and dark condition to the laboratory at the Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University.

3.3 Experimental setup

3.3.1 Sediment chamber

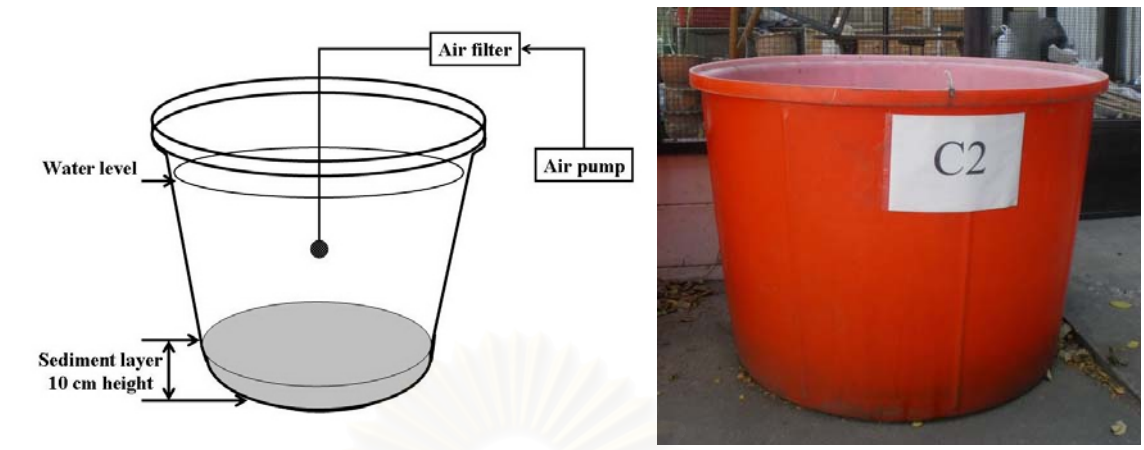
With this study, experiments were performed in sediment chambers under laboratory conditions. Sediment chamber used in sections 4.2.1 and 4.2.5 was made of transparent acrylic tube with 20 cm in diameter (0.03 m^2 surface area) and 50 cm in aerial height (Figure 3.2B). Upper part of the chamber was covered with plastic sheet to prevent contamination. Sediment sample was packed in the chamber to 10 cm height. During the experiment, aeration was continuously supplied with electric air pump with air filter and bubbling through air stone. In addition, some of the experiments in sections 4.2.3-4.2.5 and 5.2.2-5.2.3 were performed using the non-transparent chambers (Figure 3.2C). These chambers were cylindrical PVC chamber (0.01 m^2 surface area) with 11 cm in diameter and 30 cm in length. Diagram of sediment chamber is shown in Figure 3.2A. The main components of the experiment chamber included air pump (LP-40, Resun, China), air filter (Acro 50, Pall, USA), air stone, DO probe (HI91410 Hanna, Portugal) and ORP probe (HI98240 Hanna, Portugal).

(A) (B) (C)

Figure 3.2 Schematic diagram (A) and photographs (B-C) of the sediment chamber used in this study.

3.3.2 Simulated outdoor shrimp pond

For simulated outdoor shrimp pond, circular plastic tank (0.73 m^2) with 500 L in volume was used. Each experiment tank was packed with sediment from shrimp pond in Pathum Thani Province (section 3.2) to the height of 10 cm and filled with 450 L of seawater (Figure 3.3). At the middle of water column, aeration was supplied with electric air pump (LP-100, Resun, China) through air stone.



(A) (B)

Figure 3.3 Diagram (A) and photograph (B) of the 500 L outdoor simulated shrimp pond used in this study.

3.4 Analysis of physical factors in water and sediment

3.4.1 Dissolved oxygen

Dissolved oxygen (DO) (mg O_2/L) in middle water column was monitored using DO meter with integrated data logger (HI91410, Hanna, Portugal).

3.4.2 Oxidation-reduction potential

Oxidation-reduction potential (ORP) (mV) was measured in water column and at 2 cm below soil surface using ORP meter (HI98240, Hanna, Portugal).

3.4.3 Temperature

Ambient air and water temperature were monitored using NTC thermologgers (HI141, HANNA, Portugal).

3.4.4 pH

Monitoring of pH in water column was performed *in situ* using pH meter (pHtestr 30, Eutech Instruments, USA).

3.4.5 Salinity

Salinity was measured in term of practical salinity unit (PSU) using hand refractometer (Atago S-8, Japan).

3.4.6 Sediment dry weight and water content

Five-grams of wet sediment was placed on a pre-recorded weight petri dish and dried in hot air oven (Memmert, Germany) at 80^{o}C for at least 5 hours (Schinner *et al*., 1995). Thereafter, the dry sediment was cooled down in desiccators before weighed using four decimals electronic balance (BP210S, Sartorious, Germany). Percentages of dry sediment matter and water content was calculated as follows:

$$\text{Percentage of dry matter} = \frac{(A-B)\times 100}{C}$$

Where A = initial weight

B = final weight from oven

C= Initial sediment weight

$$\text{Percentage of water content} = 100 - \%drymatter$$

3.4.7 Soil texture

Percentages of sand, silt and clay in sediment were evaluated by particle size analysis by Department of Land Development, Thailand.

3.5 Analysis of chemical parameters in water and sediment

3.5.1 Inorganic nitrogen compounds (ammonia, nitrite and nitrate) and phosphorus

3.5.1.1 Sample preparation

Water sample for dissolved inorganic nitrogen and phosphorus was filtered through 1.2 µm GF/C glass-fiber filter (Whatman, USA) to remove particulate matters following by chemical analysis (3.5.1.2-5). On the other hand, pore water in sediment sample was placed in 1.7 micro-centrifuge tube separated by centrifugation at 2500 rpm for 15 minutes. Pore water (supernatant) was collected for further nitrogen and phosphorus analysis (3.5.1.2-5). For nitrogen content in sediment, one gram of sediment sample was extracted with 5 mL of 2 M KCL, shaken at 120 rpm for 30 minutes under ambient temperature, and centrifuged at 2500 rpm for 15 minutes (Schinner *et al*., 1995). Finally, the supernatant was collected for water analysis. All water samples for water, pore water and sediment analysis were practically stored in refrigerator at -20ºC prior to water quality analysis.

3.5.1.2 Determination of ammonia (NH_4^+-N)

Ammonia concentration (mg-N/L) was analyzed by Phenate method (Parson *et al*., 1989) with modification. One milliliter of filtrated water sample was placed into 1.7 mL micro-centrifuge tube and then added with 40 µL of phenol solution (dissolve 20 g of crystalline phenol in 200 mL of 95% (v/v) ethyl alcohol). For the following step, the mixture was added with 40 µL of sodium nitroprusside (dissolve 1.0 g of sodium nitroprusside ($Na_2Fe(CN)_5NO.\ 2H_2O$) in 200 mL de-ionized water) and 50 µL of oxidizing solution before mixing. The oxidizing solution was prepared by mixing alkaline reagent (dissolve 100 g of sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 5 g of sodium hydroxide (NaOH) in 500 mL of de-ionized water) and sodium hypochlorite solution (commercial hypochlorite with the concentration approximately 1.5 N), at the mixing ratio of 4:1. Reaction tube was incubated at room temperature for one hour then ammonia concentration was measured by UV-visible spectrophotometer (Genesys 10 series, Thermo Spectronic, USA) at 640 nm against standard curve of 0.1-1.0 mg-N/L NH_4Cl.

3.5.1.3 Determination of nitrite (NO_2^--N)

Nitrite was measured on the basis of colorimetrical analysis by Griess-Ilosvay Diazotization method (Strickland and Parsons, 1972) with modification. One milliliter of filtrated water sample was transferred to 1.7 mL micro-centrifuge tube and then added with 20 µL of sulfanilamide solution (dissolve 5 g of sulfanilamide and 50 mL of conc.HCl in 500 mL of dH_2O). After mixing for 1 minutes, 20 µL of N-(1-naphthyl)-ethylenediamine dihydrochloride (NNED) solution (dissolve 0.5 g of NNED in 500 mL of dH_2O) was added, mixed and incubated for at least 30 minutes prior to color development. Nitrite concentration was measured at 543 nm using UV-visible spectrophotometer against standard nitrite solution (0.1-0.4 mg-N/L $NaNO_2$).

3.5.1.4 Determination of nitrate (NO_3^--N)

Nitrate concentration (mg-N/L) was analyzed by UV screening method. Water sample was filtered through GF/C filter and measured directly by UV-visible spectrophotometer at 220 and 275 nm (APHA, 1992). Calculation of nitrate concentration was as following:

$$Nitrate(mg-N/L) = \frac{(Abs_{220nm} - Abs_{275nm}) \times A}{B}$$

Where A = concentration of nitrate in standard curve (mg-N/L)

B = absorbance of standard curve (220nm-275nm)

Standard nitrate solution was prepared using 1-10 mg-N/L of sodium nitrate. It has to be noted that, this method must be strictly used with nitrate concentration between 1-10 mg-N/L. Water sample containing high nitrate concentration (over 10 mg-N/L) can be diluted with de-ionized water prior to analysis but water containing low nitrate concentration (below 1 mg-N/L) was not applicable with this method. Moreover, high nitrite concentration can interfere with nitrate measurement hence concentration of nitrate must be subtracted with nitrite concentration.

3.5.1.5 Determination of phosphate (PO_4^{2-}-P)

Phosphorus analysis (mg-P/L) was referred to Strickland and Parsons (1972). Mixed reagent was the mixture of four solutions *i.e.* ammonium molybdate solution (dissolve 15 g of $(NH_4)_6Mo_7O_{25}.4H_2O$ in 500 mL dH_2O), sulfuric acid solution (add 140 mL of conc. H_2SO_4 in 900 mL of dH_2O), ascorbic solution (dissolve 27 g of ascorbic acid in 500 mL of dH_2O) and potassium antimonyl-tartrate solution (dissolve 0.34 g of potassium antimonyl-tartrate in 250 mL of dH_2O), at the mixing ratio of 2:5:2:1. One milliliter of water sample was added with 100 µL of the mixed reagent, homogenized and left for 2 hours reaction time. Thereafter, the sample was measured by spectrophotometer at 885 nm wavelength. Standard phosphate solution was 0.1-2.0 mg-P/L of KH_2PO_4.

3.5.2 Determination of total nitrogen in dry sediment

Method for total nitrogen analysis in sediment was modified from Gross *et al*. (1999). Dried sediment sample, 0.5 to 1 mg in dry weight, was placed into glass vial and then added with 5 mL of 0.075 N NaOH and 0.1 g of potassium persulfate ($K_2S_2O_8$). Glass vial was sealed by screw cap and autoclaved at 121ºC for 30 minutes. After cooling to the room temperature, the solution was added with 1 mL of borate buffer (dissolve 61.8 g of boric acid and 8 g of NaOH in a liter of dH_2O), mixed and centrifuged at 2500 rpm for 5 minutes. Supernatant was removed for nitrate analysis (see section 3.5.1.4).

3.5.3 Total chlorophyll analysis

Water samples were filtered through a GF/C filters for collecting phytoplankton and stored under -20ºC for total chlorophyll analysis. Concentration of total chlorophyll (mg/L) was determined by the method modified from Strickland and Parsons (1972). Five milliliters of 90% acetone was added to filter (see section 3.5.1.1). The filter in the acetone was then stored at 4ºC for 20 hours. Prior to analysis, chlorophyll extraction was enhanced by filter grinding in acetone using hand homogenizer for 3 minutes under dark and cold condition. Supernatant was separated by a centrifuge at 2500 rpm for 5 minutes. Total chlorophyll concentration was

measured by spectrophotometer at three wavelengths, 665, 645 and 630 nm. The amount of total chlorophyll concentration was calculated as follows:

$$(1) Cholophyll-a(mg/L) = \frac{C}{V}$$

Where $C = 11.64(Abs_{663nm}) - 2.16(Abs_{645nm}) + 0.10(Abs_{630nm})$

V = water sample volume (L)

$$(2) Cholophyll-b(mg/L) = \frac{C}{V}$$

Where $C = 20.7(Abs_{645nm}) - 4.34(Abs_{665nm}) - 4.42(Abs_{630nm})$

V = water sample volume (L)

$$(3) Cholophyll-c(mg/L) = \frac{C}{V}$$

Where $C = 55(Abs_{630nm}) - 4.64(Abs_{665nm}) - 16.3(Abs_{645nm})$

V = water sample volume (L)

3.5.4 Determination of organic content in dry sediment

The organic content in dry sediment (%) was determined in relation to weight loss on ignition. Dry sediment samples after regular dry weight analysis (from section 3.4.6) were weighed and subsequently placed in a furnace at 450°C for 4 hours (APHA, 1972). The organic matter was determined as the difference between the dry weight and weight after being burned in the furnace.

3.5.5 Alkalinity

Alkalinity of water (mg-CO_3/L) was measured by using a commercial test kit produced by factually of Veterinary Science, Chulalongkorn University; based on titration method.

3.6 Viable colony count method of nitrifying bacteria density in water and in sediment

3.6.1 Selective medium for nitrifying bacteria

Agar media for isolating nitrifying bacteria was modified from Schimidt and Belser (1982). One liter of medium composed of seawater (5 and 20 PSU) plus 2% agar, 10 mL of macronutrients solution (dissolve 17.416 g of K_2HPO_4, 4.928 g of $MgSO_4 \cdot 7H_2O$ and 1.472 g of $CaCl_2 \cdot 2H_2O$ in a liter of distilled water), 1 mL of micronutrients solution (dissolve 0.618 g of H_2BO_3, 1.21 g of $Na_2MoO_4 \cdot 2H_2O$, 0.238 g of $CoCl_2 \cdot 6H_2O$, 0.198 g of $MnCl_2 \cdot 4H_2O$, 0.25 g of $CuSO_4 \cdot 5H_2O$, 0.144 g of $ZnSO_4 \cdot 7H_2O$ and 0.14 g of $NiSO_4 \cdot 7H_2O$ in one liter of distilled water), 1 mL of chelate solution (dissolve 5 g of Titriplex III and 2.78 g of $FeSO_4.7H_2O$ in one liter of distilled water), 0.5 M of $NaCO_3$ and 20 g of agar. Thereafter, 4 mM of $(NH_4)_2SO_4$ or 0.2 mM of KNO_2 was added to obtain the medium for ammonia oxidizing bacteria (AOB) or nitrite oxidizing bacteria (NOB), respectively. Finally, the media were autoclaved at 121ºC for 20 minutes.

3.6.2 Preparation of bacterial sample and serial dilutions

Serial dilutions of water sample were prepared with sterilized seawater. One gram of fresh sediment was transferred into 9 mL sterilized seawater with 20 PSU and then shaken at 120 rpm for 30 minutes. Sediment suspension was diluted in series using sterilized seawater to obtain the dilution between 10^{-1} to 10^{-7}.

3.6.3 Spread plate and incubation

To count bacterial colony in agar plate, the suitable number of bacteria colony must be within the range between 30 and 300 colonies per plate. In practical, the serial dilutions between 10^{-3}, 10^{-4} and 10^{-5} were selected for colony counting. One hundred microliters of bacteria suspension was spread on agar plates with selective medium for AOB and NOB (section 3.6.1) and incubated at 28ºC for one month.

3.6.4 Determination of nitrifying bacteria morphology

Morphology of isolated nitrifying bacteria was studied by scanning electron microscope (SEM) at the Scientific and Technological Research Equipment Centre, Chulalongkorn University.

3.7 PCR-DGGE analysis for assessment bacteria community

3.7.1 Genomic DNA extraction

3.7.1.1 AOB and NOB

For bacteria colony sample, AOB and NOB colonies were obtained after being cultivated in selective medium for nitrifying bacteria (section 3.6.1). Bacteria were collected by centrifuge at 12000 rpm for 5 minutes. Supernatant was removed and bacterial pellet was stored in the freezer at -20°C for further molecular analysis. Genomic DNA was extracted using Chelex 100 resin (Bio-Rad Laboratories, USA). Bacterial pellet were mixed with 50 µL Chelex 100 resin solution (0.1 mg of resin in 1 mL final volume of 1xTE buffer), centrifuged at 2500 rpm for 5 minutes, incubated at 56ºC for one hour with gently shaking, and further incubated at 95ºC for 15 minutes. After extraction process, the extracted DNA solution was stored at 4°C prior to use.

3.7.1.2 Bacteria in water

For water sample, 500 to 1000 ml of water sample was filtered through a 0.22 µm membrane filter (GS, Millipore, UK) under aseptic condition. Filters were kept frozen at -20°C for further DNA extraction. DNA was extracted from the filter by bead-beating method using the FastDNA SPIN kit for soil (Qbiogene, USA). This kit consisted of three components: (1) lysis matrix, homogenization reagent, (2) DNA purification reagent, and (3) elution regent. After extraction, crude DNA from each sample was verified by electrophoresis with 1% agarose (Bio-Rad Laboratories, USA) and visualized by UV excitation of ethidium bromide staining.

3.7.1.3 Bacteria in sediment

For sediment sample, sediment at 2 cm vertical depth from sediment surface were collected and stored at -20°C for further DNA extraction. Sediment sample, 200-300 mg wet weight, was extracted using FastDNA SPIN kit for soil (Qbiogene, USA). Genomic DNA was verified by visualizing with agarose gel electrophoresis.

3.7.2 Polymerase chain reaction (PCR)

In this study, two primer sets were used for bacteria diversity analysis. Details of target sites, sequences and specificity of primers targeting small subunit ribosomal RNA are showed in Table 3.1.

3.7.2.1 PCR amplification of partial 16S rRNA gene for total bacterial community

The primers used for amplifying the 16S rRNA gene of bacteria were PRBA338f plus GC clamp (40bp) and PRUN518r (Table 3.1). These primers were designed for amplifying partial 16S rDNA sequences of prokaryotic bacteria with expected size at 234 bp. The PCR mixture (final volume 30 µL) was composed of 5 µL crude extracted DNA, 7 µL of sterilized dH_2O, 15 µL Taq PCR Master Mix kit (5 mL of Taq master mix contains 250 Units of Taq polymerase, 2x QIAGEN PCR buffer, 3 mM $MgCl_2$, and 400 µM of each dNTP (Qiagen, UK)), and 1.5 µL of 10 µM of each primer. The thermal cycling was carried out by an initial denaturation step at 92°C for 2 minutes, followed by 30 cycles of denaturation at 92°C for 1 minute, annealed at 55°C for 0.5 minute, and elongated at 72°C for 1 minute. The PCR was completed by a final elongation step at 72°C for 6 minutes.

3.7.2.2 PCR amplification of partial 16S rRNA gene for ammonia oxidizing β-proteobacteria community analysis

Amplification of partial 16S rDNA of AOB was performed using CTOs primer sets and the PCR product was 465 bp fragments. Details of these primer sets are showed in Table 3.1. The primers ratio of CTO 189fA/B-GC to CTO 189fC-GC (Kowalchuk *et al*., 1997) was 2:1. PCR reaction was modified from

protocol described by Kowalchuck *et al*. (1997) that each reaction (final volume, 30 µL) containing 2 µL of genomic DNA, 4 µL of sterilized dH_2O, 2 µL of each primers (10µM) and 20 µL of Taq PCR Master Mix kit (5 mL of Taq master mix contains 250 Units of Taq polymerase, 2x QIAGEN PCR buffer, 3 mM $MgCl_2$, and 400 µM of each dNTP (Qiagen, UK)). The thermal cycling was carried out by an initial denaturation step at 94°C for 2 minute, followed by 35 cycles of denaturation at 94°C for 30 sec, annealed at 55°C for 45 sec, and elongated at 72°C for 45 sec. The PCR was completed by a final elongation step at 72°C for 5 minutes.

Table 3.1 Target sites, sequences and specificity of primers targeting small subunit ribosomal RNA and expected size used for DGGE analysis.

Primer	Target site	Nucleotide sequence (5´-3´)	Specificity	Expected size	Reference
PRBA338f-GC clamp	338-357	CgC CCg CCg CgC gCg gCg ggC ggg Cgg ggg CAC ggg ggg CCT ACg ggA gg C AgC Ag	All bacteria	236-bp	Muyzer *et al.* (1993)
PRUN518r	518-534	ATT ACC gCg gCT gCT gg	All bacteria		Muyzer *et al.* (1993)
CTO189A/Bf-GC clamp	189-207	CgC CCg CCg CgC ggC ggg Cgg ggC ggg ggC Acg ggg ggA gRA Aag Cag ggg ATC g	ß-AOB	465-bp	Kowalchuck *et al*. (1997)
CTO189Cf-GC clamp	189-207	CgC CCg CCg CgC ggC ggg Cgg ggC ggg ggC Acg ggg ggA ggA AAg TAg ggg ATC g	ß-AOB		Kowalchuk *et al*. (1997)
CTO654r	633-654	CTA gCY TTg Tag TTT CAA ACg C	ß-AOB		Kowalchuk *et al*. (1997)

3.7.3 DGGE analysis of amplified partial 16S rRNA gene

DGGE profiles were generated for the comparison of bacterial communities during the experimental period. The DGGE analysis was performed using the DCode universal mutation detection system (Bio-Rad Laboratories, USA).

3.7.3.1 Separation of 236 bp nucleotide fragments amplified by PRBA338f-GC and PRUN518r primer

Twenty five microliters of PCR products were mixed with 5 µL of 6x loading dye (Bio-Rad Laboratories, USA) and then run on 8% of polyacrylamide gradient gel (16x16 cm of gel size and 1 mm of gel thick) made by a gradient maker (Bio-Rad Laboratories, USA) according to the manufacturer's guidelines. The denaturing gradient range was 30-60% (Muyzer *et al*., 1993). To prepare the gradient gel, 80% denaturing gel solution (20 mL of a 37.5:1 acrylamide-bisacrylamide solution, 2 mL of 50xTAE buffer, 33.6 g of Urea, 32 mL formamide and adjust volume to 100 mL with dH_2O) was mixed in various proportion with 0% denaturing gel solution (20 mL of a 37.5:1 acrylamide-bisacrylamide solution, 2ml of 50xTAE buffer and adjust volume to 100 mL with dH_2O) in order to obtain the denaturing gradient acrylamide gel with 30 to 60% denaturant. The gel was polymerized by 0.09% of TEMED and 10% of ammonium persulfate. Before polymerization occurred, 3 ml stacking gel without denaturant was added on top of the gradient gel. After DNA loading, DGGE was run at 130 V for 6 hours in 1xTAE buffer at constant temperature (60ºC). After that, the gel was stained with ethidium bromide solution (dissolve 4 µL of 10 mg/mL ethidium bromide in 50 mL dH_2O) for 20 minutes and visualized in gel documentation instrument (Dolphin-Doc Plus, USA).

To obtain high resolution DGGE patterns, some of DGGE gels were stained using Silverstar Staining kit (Bioneer, Korea). Before casting the DGGE gel, the short glass plate was treated with a binding solution to chemically cross-link the gel to the glass plate. This step is essential to prevent tearing of the gel during the staining procedure. The long glass plate was coated with the glass coat solution. The staining protocol was started by fix the gel in 750 mL of the fix/stop solution (10% of gracial acetic acid) and agitate well for 30 minutes, immerge in 750 mL of 0.1% of enhancing solution and agitate well for 30 minutes, rinse 2 times with ultrapure water

(for 3 minutes each) and stain in 750 mL of the staining solution and agitate well for 30 minutes. Then the gel was washed with ultrapure water and placed immediately into 750 mL of the chilled developing solution. Agitate the gel until all DNA bands appeared; thereafter add 750 mL of the fix/stop solution directly to the developing solution and incubated with shaking for 4-5 minutes to terminate the developing reaction. The gel was rained in ultrapure water for 5 minutes and then placed at the room temperature. Gel visualization was using a light box (SLB-7, Samsung, Korea). Image of the gel was photographed using a digital camera.

3.7.3.2 Separation of 465 bp nucleotide fragments amplified with CTOs primers for nitrifying bacteria

Twenty five microliters of PCR products was separated in 8% polyacrylamide gel with 30-60% denaturing gradient. The gradient gel was obtained by mixing 80% denaturant containing 5.6 M urea and 32% formamide with 0% denaturant acrylamide solution (see in section 3.7.3.1). The DGGE was run under 75 V for 16 hours in 1xTAE buffer at 60 ºC constant temperature. The gel was finally stained with a Silverstar Staining kit (Bioneer, Korea) for DNA analysis (section 3.7.3.1).

3.8 PCR-DGGE analysis for eukaryotic microorganism community assessment

3.8.1 DNA extraction

Phytoplanktons including other suspended eukaryotic microorganisms from water sample were collected through 1.2 µm GF/C glass-fiber filter (Whatman, USA). Plankton cells were removed from the filter by eluting with 1xTE buffer, centrifuged at 12000 rpm for 5 minutes. Supernatant was removed and cells pellet was stored at -20ºC prior to DNA analysis. For genomic DNA extraction, plankton cells were disrupted by ultrasonication (Sonic Materials Inc, USA) at 20 watt for 1 minute on ice. Extraction of genomic DNA was performed using a Genomic DNA Extraction kit for Plant (RBC bioscience, Taiwan). This kit contained of four

components: (1) lysis buffer, (2) RNase A, (3) Washing buffer and (4) DNA elution buffer. Extracted DNA was checked by agarose gel electrophoresis.

3.8.2 PCR amplification of the partial 18S rRNA gene

Amplification of crude DNA extract was performed using conserved primers 1427f-GC clamp (5´-CgC CCg CCg CgC CCC gCg CCC ggC CCg CCg CCC CCg CCC CAT CTg TgA TgC CCT TAg ATg TTC Tgg g-3´) and 1616r (gCg gTg TgT ACA AAg ggC Agg g). The primers were specific for 18S rDNA of eukaryotic aquatic organisms (van Hannen *et al*., 1998). This primer set generated 250 bp of PCR products. PCR amplification was according to the method modified from van Hannen *et al*. (1998) that each reaction (final volume, 30 µL) containing 5 µL of genomic DNA, 5 µL of sterilized dH_2O, 2 µL of bovine serum albumin (200 ng/µL), 1.5 µL of each primers (10µM) and 25 µL of Taq PCR Master Mix kit (5 mL of Taq master mix contains 250 Units of Taq polymerase, 2x QIAGEN PCR Buffer, 3 mM $MgCl_2$, and 400 µM of each dNTP (Qiagen, UK)). The thermal cycling was carried out by an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 0.5 minute, annealed at 52°C for 1 minute, and elongated at 68°C for 1.5 minute. The PCR was completed by a final elongation step at 68°C for 10 minutes.

3.8.3 DGGE analysis of the partial 18S rRNA gene amplified from eukaryotic plankton

The 250 bp of nucleotide fragments generated by primers pair 1427f-GC and 1616r was analyzed using the DGGE DCode universal mutation detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The polyacrylamide gradient gel (16x16 cm of gel size and 1 mm of gel thick) was composed of 8% of acrylamide:bisacrylamide mixture ratio at 37.5 in 1xTAE buffer. The 30–60% denaturants were generated with the aid of a gradient maker (Bio-Rad Laboratory, USA). After that, the gel was run at 200 V for 6 hours in 1xTAE buffer at 60ºC constant temperature. Last, the gel was stained by using a Silverstar Staining kit (Bioneer Inc, Korea)

3.9 Recovery of DGGE bands and analysis of DNA sequencing and DGGE banding

3.9.1 Recovery of DGGE bands

The selected DNA bands of interest were cut out from denaturing gradient gels with a razor blade sterilized with 70% ethanol. Each single DNA band was placed into a 1.7 microcentrifuge tube. These tubes was added with 30 µL sterilized dH_2O and incubated overnight at 4ºC.

3.9.2 DNA sequencing analysis

The selected bands from a DGGE gel were cut out and 16S rDNA was re-amplified with forward primer without GC clamp. These PCR products were purified by using Qiaquick PCR preps (Qiagen, USA) and directly sequenced (Tech Dragon Limited, Hong Kong; www.techdragon.com.hk). DNA sequences were analyzed using the Seqmatch program in the Ribosomal Database Project II (RDP II) (http://rdp.cme.msu.edu/). If the sequence had low similarity in RDP II database, the closest matching to each sequence was further analyzed with the BLASTN in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov).

3.9.3 Phylogenetic tree analysis

Distance matrix of DNA sequences was performed with a Jukes-Cantor model. Regarding to RDP II database, 16S rRNA sequences were aligned with the nearest neighbors. Finally, the phylogenetic tree was constructed by neighbor-joining method.

3.9.4 Shannon-Weaver diversity index

Numbers of DNA bands and their relative intensities within each lane in the gel were counted (Quality One software, Bio-Rad Laboratories, USA). This data was used for calculating the diversity indices, species richness, evenness and the

Shannon-Weaver index (Shannon and Weaver, 1949). Species richness (*R*) was determined from the number of bands in each lane. The Shannon-Weaver index (*H*) was calculated as follows:

$$H = -\sum(P_i)(\log P_i)$$

Pi is the important probability of the bands in each lane, which calculated from *ni/N*, *ni* is the peak height of a band and. *N* is the sum of all peak heights in each lane.

Species evenness (*E*) was calculated by using the equation (Eichner *et al*., 1999):

$$E = \frac{H}{\ln(R)}$$

3.9.5 Cluster analysis

Similarity between the DNA band patterns generated by PCR-DGGE of each samples was analyzed using Nei and Li's coefficient (95% confidence). The UPGMA (unweighted pair-group method with arithmetic averages) was used for cluster analysis and dendrogram generation. The UPGMA was carried out using the BIO-1D software (Vilber Lourmat, France).

CHAPTER IV

EFFECT OF ENVIRONMENTAL CONDITIONS ON INORGANIC NITROGEN CONVERSION AND BACTERIAL DIVERSITY IN SEDIMENT FROM SHRIMP POND UNDER LABORATORY CONDITION

4.1 Introduction

In aquaculture pond, a traditional practical procedure for nitrogen waste removal is water exchange. Release of wastewater containing high nitrogen concentration from aquaculture pond is among the most concern as an environmental problem in natural water resource. Law, regulations and standards from governmental and private organizations have been announced to promote the environmental friendly aquaculture activities. Nitrogen waste management is one of the most important practices of aquaculture system. Nitrogen cycle in earthen aquaculture pond is rather complex process which involved with many biological components. Release of ammonia-nitrogen from aquatic animals has been well studied. It is known that approximately 75% of nitrogen input from feed into the aquaculture system is released into the water (Hargreaves, 1998). However, several pond dynamic processes such as natural release of ammonia-nitrogen from sediment and roles of sediment as the natural treatment of inorganic nitrogen compounds are rarely studied.

This study was an attempt to enhance nitrogen conversion by bacterial activities in shrimp pond sediment under laboratory condition. Study on effect of environmental factors *i.e.* salinity, dissolved oxygen, inorganic carbon (sodium bicarbonate), organic carbon (methanol) and illumination on bacterial nitrogen removal processes and monitoring of bacterial diversity using PCR-DGGE technique (Muyzer *et al.*, 1993; Trevors, 1998; Hill *et al.*, 2003) were accomplished. Finally, an alternative sediment treatment by enhanced nitrification-denitrification process was proposed.

4.2 Materials and methods

4.2.1 Experimental units

The experiments in this chapter were performed in sediment chambers under laboratory condition. The detail of sediment chambers was described in section 3.3.1 and also the diagram of the experimental chamber was given in Figure 3.2. All chambers were packed at 10 cm height with sediment from shrimp pond (section 3.2). Experiments in section 4.2.2 and 4.2.6 were performed using transparent acrylic chambers with 0.03 m^2 surface area. Other experiments including section 4.2.3 through 4.2.5 were evaluated in the non-transparent PVC chambers with 0.01 m^2 surface area.

4.2.2 Effect of salinity on inorganic nitrogen conversion and bacterial diversity in pond bottom soil from shrimp pond

4.2.2.1 Experiment design

To study effect of salinity on inorganic nitrogen conversion, two treatments, each with two replicates sediment chambers, were assigned in this experiment. There were (1) sediment chambers filled with the low salinity seawater (5 PSU) and (2) sediment chambers filled with the high salinity seawater (20 PSU). Seawater volume of both chambers was 8 L. Alkalinity in water was adjusted to 120 mg/L. All experimental chambers were continuously aerated and incubated at ambient air under dark condition. Detail of sediment chamber setup was described in section 3.3.1.

4.2.2.2 Water and sediment quality analysis

Sediment dry weight, water content, soil texture and organic content in sediment sample from shrimp pond were analyzed as mention in section 3.4.6-7 and 3.5.4. Ammonia, nitrite and nitrate in water and in sediment from shrimp pond were also monitored using the methods described in section 3.5.1.

During 31 days of the experiment, water samples were collected daily while sediment samples were collected weekly for water and sediment

analysis. Water, pore water and extracted sediment samples were analyzed for ammonia (Phenate method; Parsons *et al*., 1989), nitrite (modified Griess-Ilosvay Diazotization method; Strickland and Parsons, 1972), nitrate (UV screening method; APHA, 1992) and phosphate (Ascorbic acid method; Strickland and Parsons, 1972). Organic content of sediment (APHA, 1992) was analyzed at the beginning and the end of the experiment.

4.2.2.3 Density, morphology and identification of nitrifying bacteria in water and in sediment

Density of nitrifying bacteria *i.e.* ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in both water column and sediment were evaluated weekly by using colony plate counting technique in selective agar medium (section 3.6.1 through 3.6.3). Morphology of isolated nitrifying bacteria was studied by scanning electron microscope (SEM).

PCR-DGGE technique was used in order to identify the AOB and NOB colonies isolated by selective medium for nitrifying bacteria. Gemonic DNA was extracted, amplified using primers PRBA338f plus GC clamp and PRUN518r (Muyzer *et al*., 1993; section 3.7.1.1 and 3.7.2.1) and separated by DGGE. Thereafter DNA band from each lane were cut, re-amplified, purified and sequenced. The closet matching to each sequences obtained from this study was analyzed using the BLASTN in the NCBI website (www.ncbi.nlm.nih.gov).

4.2.2.4 Sediment bacterial diversity

At the initial day and the final day, bacterial diversity at the top 2 cm of sediment was analyzed by PCR-DGGE technique, as described in section 3.7.1.3, 3.7.2.1 and 3.7.3.1. Seven dominant bands from the DGGE gel were cut, re-amplified, purified and sequenced. Sequences obtained from this study were analyzed using the Seqmatch in the RDP II website (http://rdp.cme.msu.edu/) and the BLASTN in the NCBI website. Phylogenetic tree was constructed by neighbor-joining method (see in section 3.9.3). Nucleotide sequences reported in this study were deposited in the NCBI nucleotide sequence databases under accession numbers FJ665399 through FJ665405.

4.2.3 Effect of dissolved oxygen on inorganic nitrogen conversion and bacterial diversity in pond bottom soil from shrimp pond

4.2.3.1 Experimental design

Sediment was packed in a non-transparent chamber (0.01 m^2), as described in section 3.2 and 3.3.1. Seawater with 20 PSU (1.4 L) was gently filled into the sediment chamber following by total alkalinity adjustment to 120 mg/L using sodium bicarbonate. There were two groups of experimental unit and each unit was performed in triplicate. Control chambers were continuously supplied with gentle air bubble to maintain DO at below 2.5 mg-O_2/L while treatment chambers were supplied with strong air bubble to obtain saturate DO concentration over 7 mg-O_2/L. At day 28, 10% methanol solution was added in to all experimental chambers to simulated the organic carbon addition at C:N ratio to 4:1.

4.2.3.2 Water quality and sediment analysis

During 35 day incubation, inorganic nitrogen *i.e*. ammonia, nitrite and nitrate in water column, pore water and extracted sediment were monitored (section 3.5.1.1-4). Total nitrogen and organic matter were detected by method in section 3.5.2 and 3.5.4.

4.2.3.3 Sediment bacterial diversity

Sediment samples were collected at the beginning, day 28 and day 35 of the experiment for PCR-DGGE analysis. In brief, genomic DNA was extracted (section 3.7.1.3). The partial 16S rRNA gene fragment was amplified (section 3.7.2.1) and separated in DGGE gel (section 3.7.3.1). With the DGGE banding patterns, UPGMA was constructed using Nei and Li's coefficient (95% confidence) in the BIO-1D software (Vilber Lourmat, France). Species richness, evenness (Eichner *et al*., 1999) and the Shannon-Weaver index were calculated (Shannon and Weaver, 1949) as described in section 3.9.4.

4.2.4 Effect of inorganic carbon addition on inorganic nitrogen conversion and bacterial diversity in pond bottom soil from a shrimp pond

4.2.4.1 Experimental design

Sediment from shrimp pond was packed in sediment chambers (section 3.3.1), filled with 1.4 L of 20 PSU seawater, continuously aerated and incubated under an ambient air condition. The experimental unit consisted of three control and treatment chambers. The treatment chambers were added with 24.36 mg-C/L (0.105 g-$NaHCO_3$/L) that provided the C:N ratio of 2:1 while control chambers had no inorganic carbon addition. The experimental period was 29 days.

4.2.4.2 Water quality and sediment analysis

Inorganic nitrogen compounds (ammonia, nitrite and nitrate) in water were measured daily while those in pore water and in extracted soil were occasionally measured (section 3.5.1.2-4). At day 0, 9, 21 and 29, total nitrogen in sediment was measured by digestion method with modification (Gross *et al*., 1999), as mentioned in section 3.5.2.

4.2.4.3 Sediment bacterial diversity

At day 0 and 29, bacteria diversity in sediment chambers was analyzed using PCR-DGGE technique (section 3.7.1.3, 3.7.2.1 and 3.7.3.1). According to the DGGE banding patterns, the UPGMA was carried out by method in section 3.9.5 and the diversity indices *i.e.* species richness, evenness and the Shannon-Weaver index were calculated (section 3.9.4).

4.2.5 Effect of organic carbon addition on inorganic nitrogen conversion and bacteria diversity in pond bottom soil from a shrimp pond

4.2.5.1 Experimental design

This experiment consisted of (1) control: sediment chambers without organic carbon addition, (2) treatment-1: sediment chambers supplemented with methanol at C:N ratio of 2:1, and (3) treatment-2: sediment chambers

supplemented with methanol at C:N ratio of 4:1. All treatments were performed in triplicate. Diagram and photograph of experimental chambers are in Figure 3.2A-B. The experiment was carried out for 48 days.

4.2.5.2 Water quality and sediment analysis

During the experiment, water and sediment samples were collected and analyzed in order to monitor the changes of inorganic nitrogen compounds (section 3.5.1.2-4). Sediment samples were digested and analyzed for total nitrogen (Gross *et al*., 1999; section 3.5.2) and burned in a furnace to determining organic matter (APHA, 1972; section 3.5.4).

4.2.5.3 Sediment bacteria diversity

At the initial day and day 48, bacteria community in sediment samples were evaluated using PCR-DGGE technique (Muyzer *et al*., 1993). DNA similarity in each sample was evaluated the UPGMA (section 3.9.5). The diversity indices were calculated by the equation given in section 3.9.4. Dominant DNA bands were cut, re-amplified, purified and sequenced (section 3.9.1-2). Sequences in this study were submitted to GenBank under accession numbers, FJ386435 through FJ386437 and FJ665406 through FJ665419.

4.2.6 Effect of illumination on inorganic nitrogen conversion of sediment from a shrimp pond under laboratory conditions

4.2.6.1 Experimental setup

The sediment from shrimp pond was packed into the transparency sediment chambers (Figure 3.2A-B). The chambers were then filled with 8 L of 20 PSU seawater and adjusted the alkalinity to 120 mg/L using sodium bicarbonate. Aeration was supplied continuously through 0.22 μm filter-disc. This study was composed of two experimental units: (1) the light chambers which were sediment chambers continuously illuminated with florescent lamp providing 1000 lux light intensity and (2) the dark chambers which were similar to light chambers except being covered with black plastic sheet to prevent light exposure. All chambers were incubated under ambient temperature between 28.6-31.5˚C. Loading of organic

matter into the sediment chambers was simulated by an addition of shrimp feed pellet at was 0.33, 0.67 and 1.67 g on day 16, 27 and 32, respectively.

4.2.6.2 Water and sediment analysis

During 46 day experiment, water was daily sampling for ammonia, nitrite and nitrate analysis (section 3.5.1.2-4). Total chlorophyll (section 3.5.3) in water column was analyzed in order to evaluate changes of phytoplankton density in the chamber.

4.2.6.3 Eukaryotic plankton diversity

At day 46, water samples from light chambers were filtered through a GF/C filter to collect phytoplankton cells. Crude DNA of phytoplankton was extracted using a genomic DNA extraction kit for plant (RBC Bioscience). Fragments of the partial 18S rRNA gene were amplified using a forward primer 1427 plus GC clamp (40bp) and a reverse primer 1616 (van Hannen *et al*., 1998) (section 3.8.2.1-3). DGGE analysis was performed in 8% (w/v) polyacrylamide gel with denaturing gradients from 30% to 60% with D-Code system (Bio-Rad Laboratory, USA), as described in section 3.8.3.

4.3 Results

4.3.1 Water and sediment quality of shrimp pond sediment

During sediment sampling from a shrimp pond in Pathum Thani Province, parameters including salinity, pH, DO, total alkalinity, and ORP in water and sediment were measured and the data is given in Table 4.1. Concentrations of inorganic nitrogen in water and in sediment are shown in Table 4.2. According to sediment analysis, water content in wet sediment was 34.50±4.47 percent and organic matter in dry sediment was 10.32±0.51 percent. Sediment texture was composed of 58% clay, 36% slit and 6% sand.

Table 4.1 Physical parameters in a shrimp pond at Phathum Thani Provience, Thailand.

Parameters	In water column	In sediment
Salinity (PSU)	5	Nd*
pH	8.25	5.04
DO (mg/L)	6.3	Nd*
ORP (mV)	97.6	-117.6
Total alkalinity (mg/L)	200	Nd*

Remark: Nd* was no detection. All parameters were measured at 15:30 h.

Table 4.2 Inorganic nitrogen in water, pore water and extracted sediment from shrimp pond.

Nitrogen compounds	In water column (mg-N/L)	In pore water (mg-N/g wet sediment)	In extracted sediment (mg-N/g wet sediment)
Ammonia	0.31±0.12	1.13±0.12	10.61±1.30
Nitrite	0.27±0.02	0.27±0.01	0.07±0.00
Nitrate	1.87±0.21	3.52±0.25	2.43±0.10

4.3.2 Effect of salinity on inorganic nitrogen compound conversion and bacteria diversity in pond bottom soil from a shrimp pond

Regarding to DO in water column and ORP in sediment layer of the sediment chambers, the results revealed that DO in water was constant at the high concentration of 7.8±0.4 mg-O_2/L while ORP in sediment ranged from -121.3 mV at the beginning day to -333.3 mV at day 8 of the experiment. After day 8, ORP was constant at –356.2±10.9 mV (Figure 4.1). Water and ambient air temperature during the experiment, as shown in Figure 4.2, was rather constant at 28±31˚C and water temperature was slightly lower than air temperature.

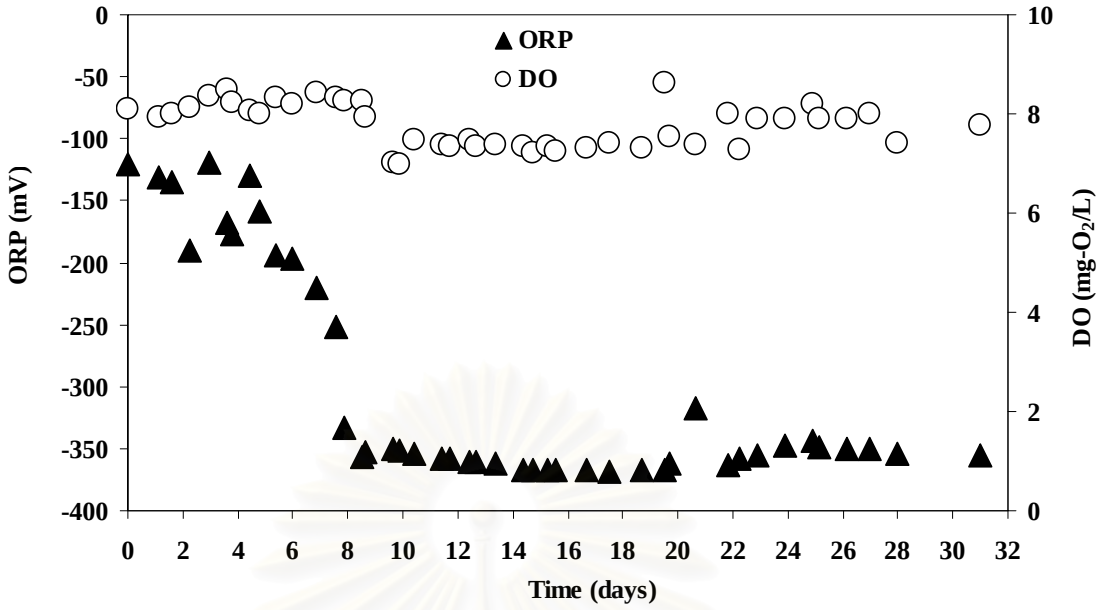


Figure 4.1 Variation of DO in the middle of a water column and ORP in sediment (at 2 cm depth) in sediment chambers throughout the experimental period.

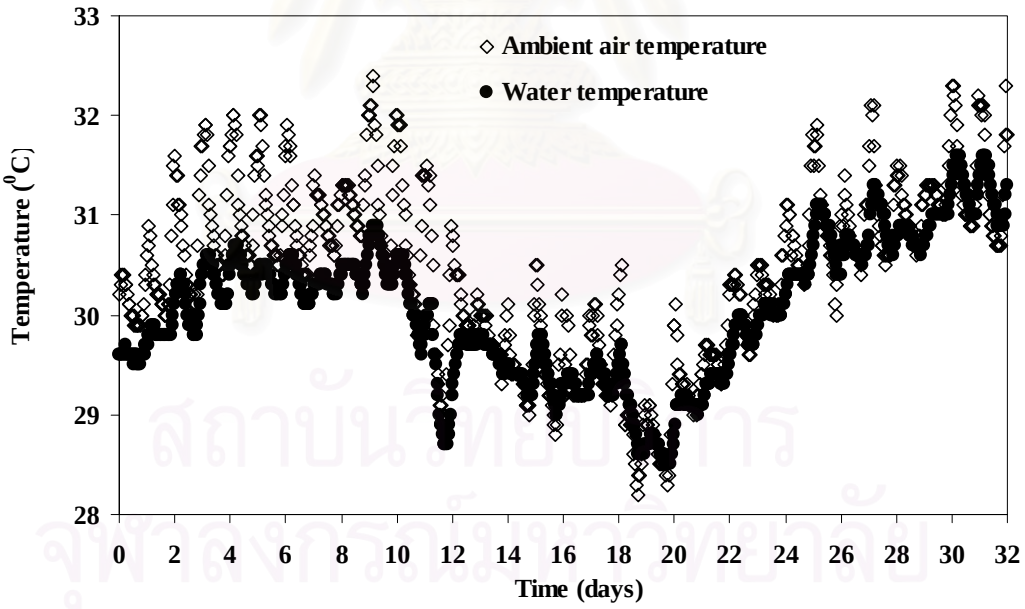


Figure 4.2 Temperature of water in the sediment chambers and ambient air during the experimental period.

4.3.2.1 Water and sediment quality

Changes of inorganic nitrogen compounds in water column were shown in Figure 4.3 (A-C). It was found that, during day 0 and day 7, ammonia in the low salinity (5 PSU) chamber gradually released to the water with the highest concentration at 0.75±0.07 mg-N/L following by a decrease to 0.05 mg-N/L at the final day. During day 7 to day 11, a peak of nitrite indicating an oxidation process of ammonia to nitrite was observed. With this process, nitrite was gradually increase to the highest concentration (0.43±0.00 mg-N/L) at day 9 then nitrite was decrease to 0.04 mg-N/L at day 13 (Figure 4.3B). Increase of nitrate was found after day 7 of the incubation period, indicating that nitrification process was complete since nitrate is the final product of nitrification. Concentration of nitrate was rather constant during day 7 to day 31 at 1.13 to 1.65 mg-N/L (Figure 4.3C). Trend of ammonia and nitrite conversion of the low (5 PSU) and high (20 PSU) salinity chambers was similar. In contrast, concentration of nitrate in high salinity sediment chamber was higher and that found in low salinity chambers.

As shown in Figure 4.4A-C, ammonia and nitrate in pore water and in extracted soil were found fluctuate with high range. Nitrite was constant at below 11.89 µg-N/g wet sediment. Trend of inorganic nitrogen fluctuation in sediment of low and high salinity chambers were similar. With high salinity chamber, the highest ammonia (75.24±0.17 µg-N/g wet sediment) was observed in extracted soil at day 12; thereafter, a decrease in ammonia was observed. With low salinity chamber, the results showed that nitrate remained at the high level (55.99 µg-N/g wet sediment) after day 2 of the experiment. The results also indicated that decomposition process reduced sediment organic content in the low salinity chamber from 15.86% to 7.87% and in the high salinity chamber from 16.57% to 8.97% within 31 days (Figure 4.5).

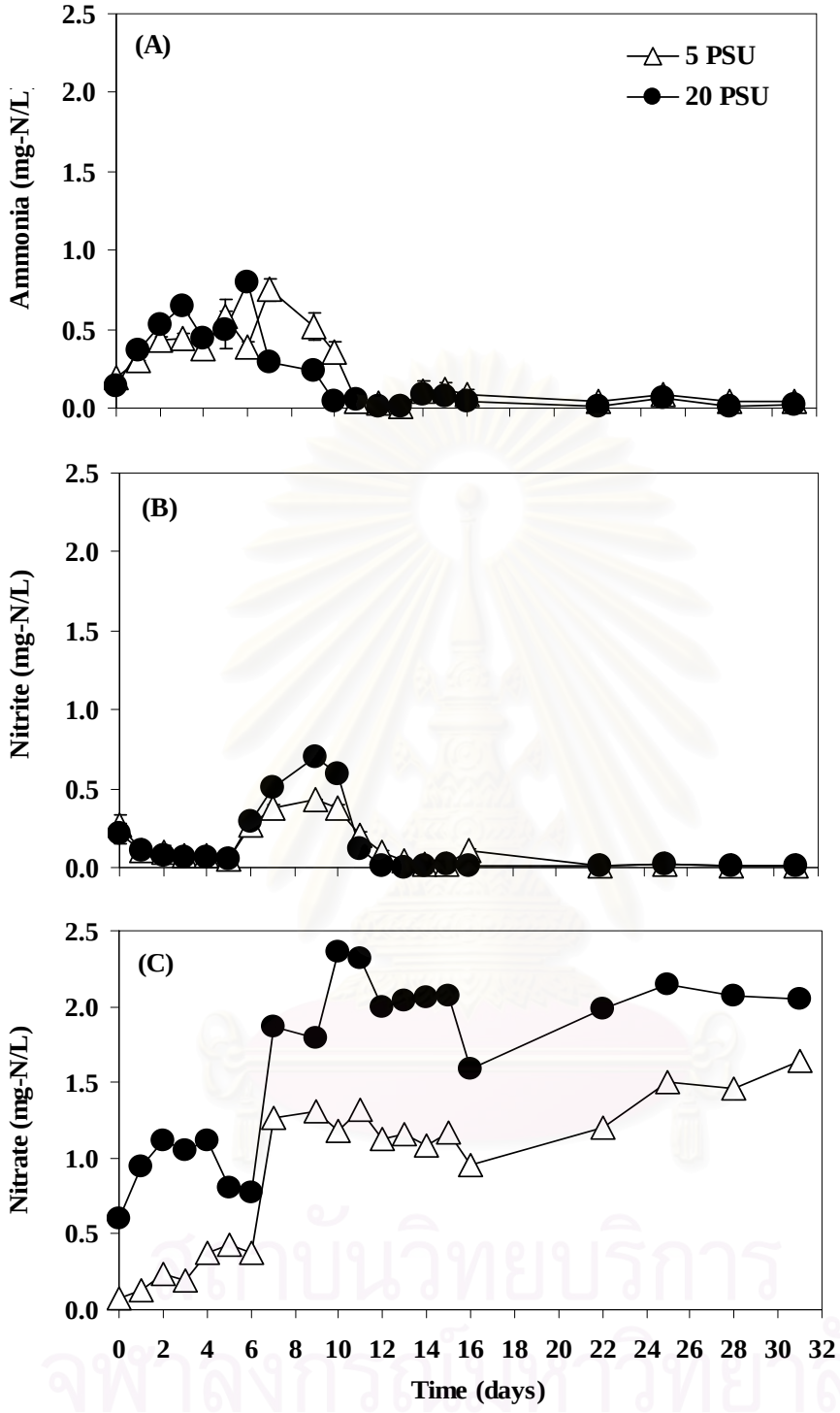


Figure 4.3 Changes of ammonia (A), nitrite (B) and nitrate (C) in the water of sediment chambers incubated with different salinity.

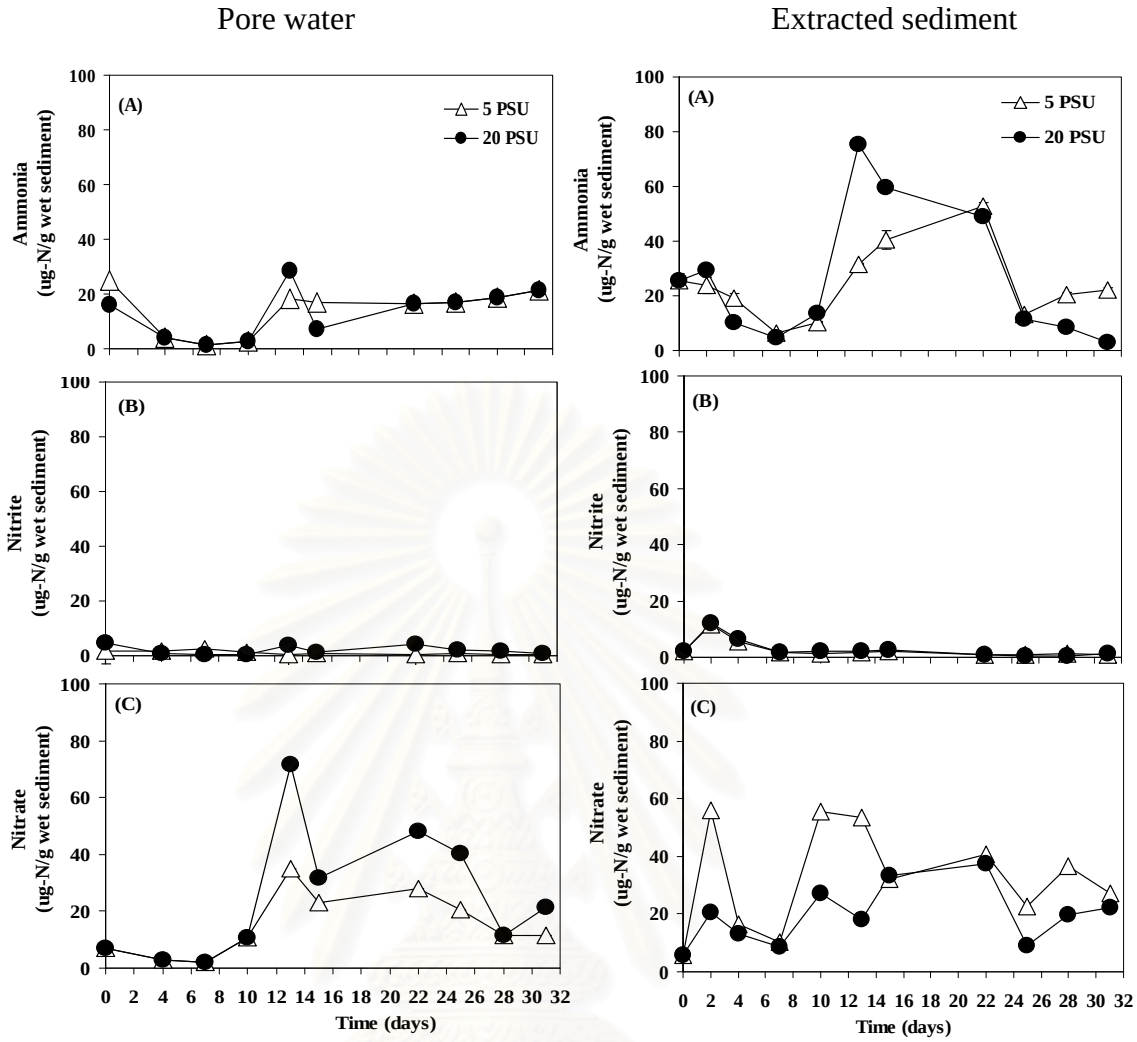


Figure 4.4 Changes in ammonia (A), nitrite (B) and nitrate (C) in pore water and extracted sediment from sediment chambers incubated in different salinity.

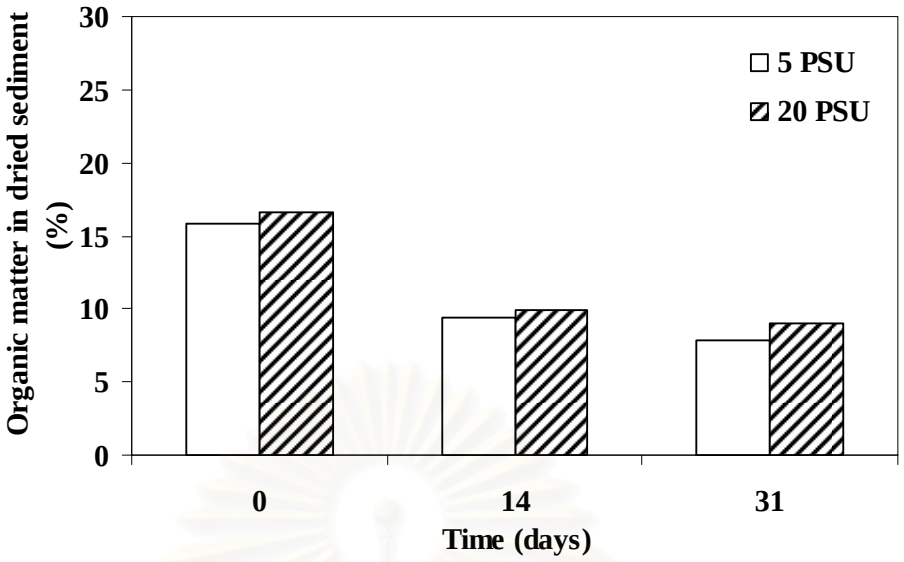


Figure 4.5 Changes in organic matter in dry sediment during a 31 day experiment.

4.3.2.2 Density and identification of nitrifying bacteria in water and in sediment

According to the colony counting method in selective media, density of both AOB and NOB in the low and high salinity chambers was steadily low in water column. On the other hand, high density of AOB and NOB was found at sediment surface. Increase of AOB and NOB in sediment was detected during the experiment (Figure 4.6A-B). The results revealed that after 7 day incubation, AOB in sediment from the low and high salinity chambers raised from 20 to $729x10^4$ CFU/g wet sediment and from 12 to $504x10^4$ CFU/g wet sediment, respectively. Growth of NOB in sediment of both chambers rose to the highest density at day 31. In the low salinity chamber, number of NOB increased from 35 to 681 $x10^4$ CFU/g wet sediment and that in the high salinity chamber was from 64 to 281 $x10^4$ CFU/g wet sediment.

Observation using SEM illustrated that bacteria isolated by AOB selective medium was mainly rod-shaped bacteria while bacteria isolated by NOB selective medium was a mixture of rod-shape and filamentous bacteria (Figure 4.7A-F). Some of AOB and NOB colonies were picked up for identification using PCR-DGGE analysis. However, the results from 16S rDNA sequence in Table 4.3 showed that all of AOB and NOB colonies as isolated by selective culture medium were not autotrophic nitrifying bacteria.

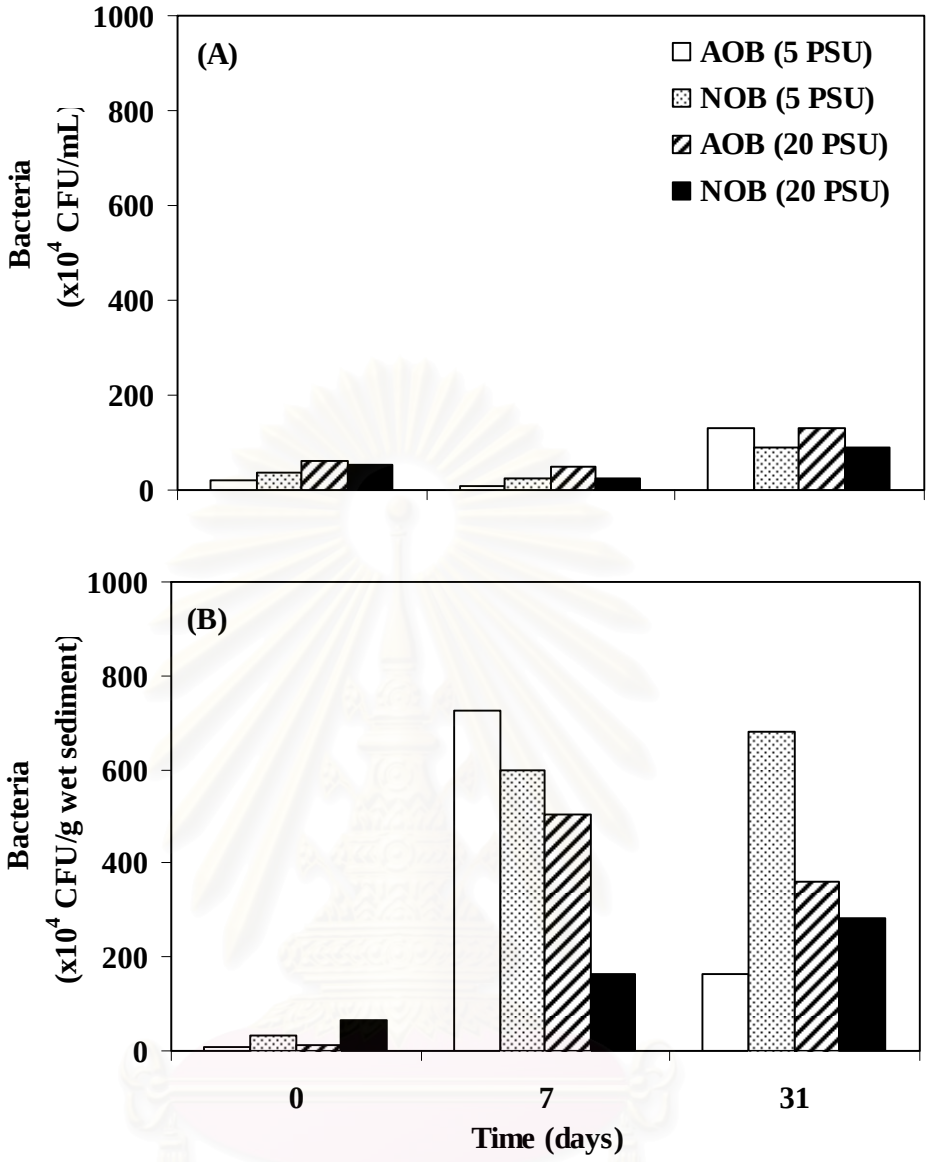


Figure 4.6 Density of nitrifying bacteria in water (A) and sediment (B) of the sediment chambers filled with 5 and 20 PSU seawater.

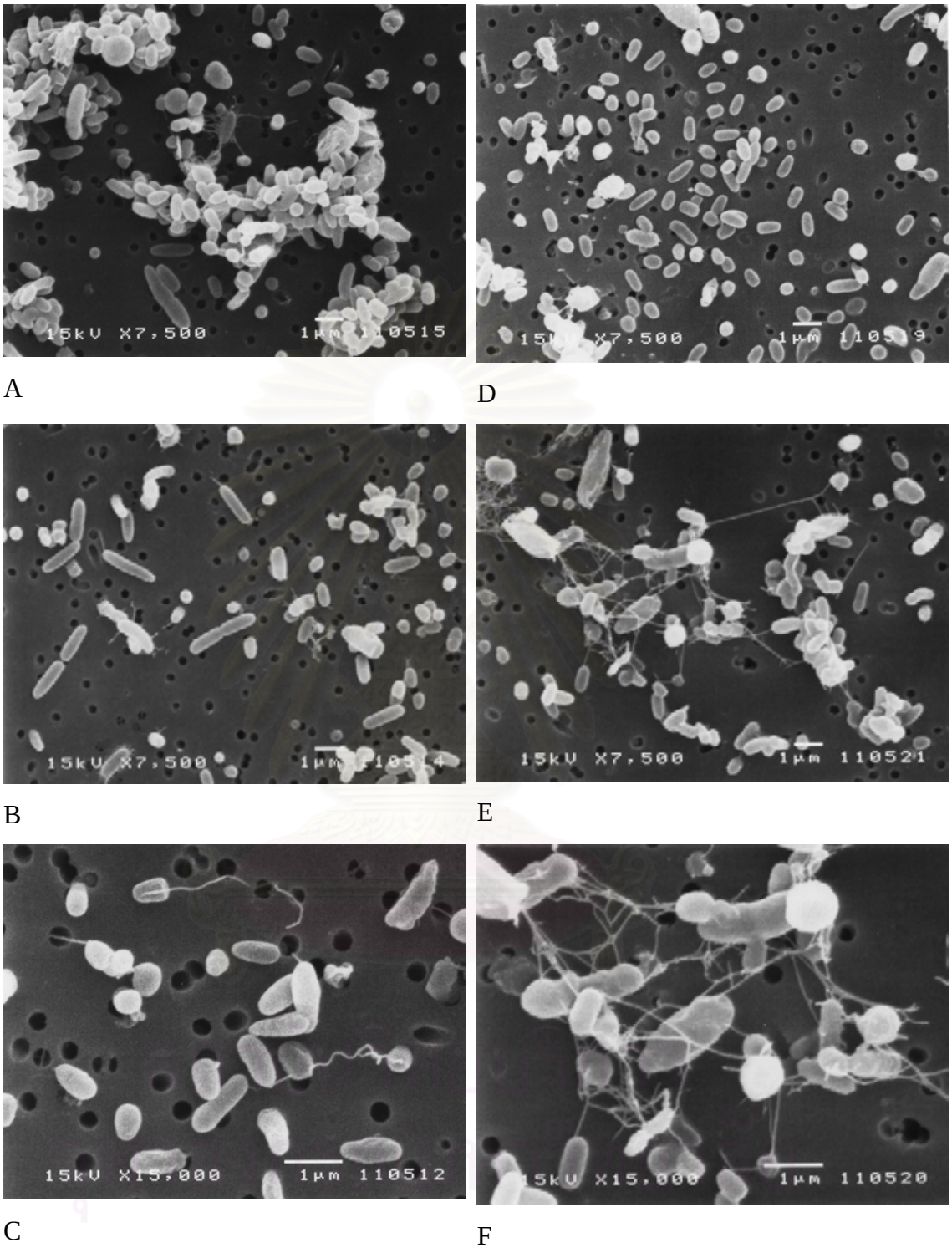


Figure 4.7 Scanning electron microscope (SEM) images of AOB (A-C) and NOB (D-F) isolated from sediment chambers using selective medium for nitrifying bacteria.

Table 4.3 Description of sequences amplified form AOB and NOB colonies.

Sample	The closets related	Accession number	Similarity (%)
AOB-1	*Bacillus* sp. Bt 10	AJ971862.1	91
AOB-2	Bacterium 4B-13	EU151853.1	94
AOB-3	*Micrococcus* sp. SeaH-As10s	FJ607363.1	95
NOB-1	*Micrococcineae* bacterium BF 23	FM173281.1	96
NOB-2	Uncultured bacterium clone h14	EU438873.1	93
NOB-3	*Bacillaceae* bacterium CL 718	FM174188.1	91

4.3.3.3 Bacterial diversity in sediment

In this study, sediment samples were collected only from the high salinity chamber (20 PSU). The DGGE profile of 16S rDNA showed that bacteria community in the sediment distinctly changed after 31 day incubation (Figure 4.8). There were at least 8 major bacteria species as indicated by dense DGGE bands at the initial day. At the final day, there were at least three new dominant bacteria (BAC-5 through BAC-7), but some dominant bacteria in the initial day *i.e.* BAC-1 through BAC-4 was disappeared. Seven interesting bands (BAC-1 through BAC-7) were then collected for further analysis.

Sequences of nucleotide fragments (BAC-1 through BAC-7) obtained from this study were compared with 16S rDNA sequence information of bacteria listed in the RDP II and the GenBank databases. The result is provided in Table 4.4. It was found that, similarity of 16S rDNA as analyzed in RDP II database were between 60% to 88% while that analyzed in the GenBank was between 91% to 99%. Some of dominant bacteria in a sediment chamber were uncultured bacteria. At the beginning day, the dominant species were *Marinobacter* and *Pseudomonas* but after 31 days the dominant species were changed to *Bacillus boroniphilus*, *Halomonas variabilis* and *Escherichia coli*. In Figure 4.9, only BAC-5 was clustered to the order

Bacillales while BAC-1 to BAC-4 and BAC-6 to BAC-7 were classified to order γ-proteobacteria.

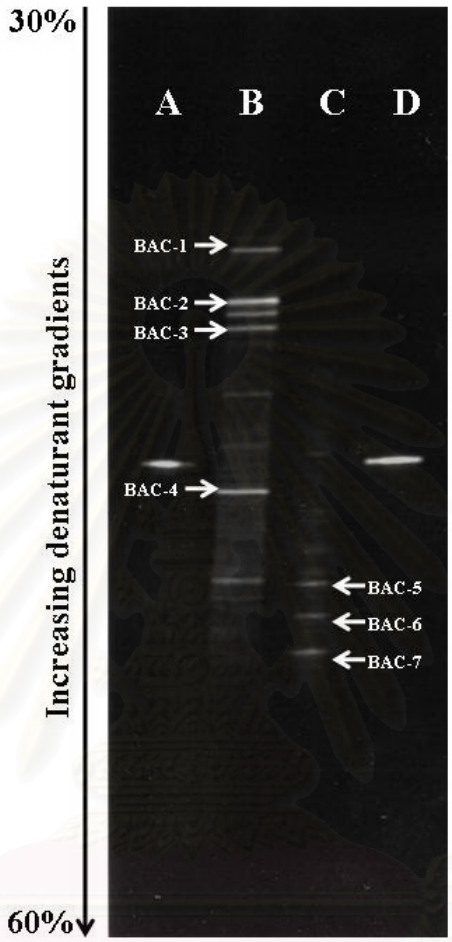


Figure 4.8 16S rDNA DGGE banding patterns of bacteria community in a sediment layer at 2 cm depth. Sediment was collected from the high salinity chamber filled with 20 PSU seawater. Lane A and D was the reference band of *E. coli* JM109; Lane B was the sediment sample from the chamber at the beginning day and Lane C was the sediment sample from the chamber at the final day.

Table 4.4 Similarity analysis of the interesting DNA bands appeared in the DGGE gel in Figure 4.8.

DNA band	RDP II database			NCBI database		
	ID	Description	Identities (%)	ID	Description	Identities (%)
BAC-1	AB026946	*Marinobacter* NK-1	60	EF444734.1	Uncultured bacterium clone Thp_B_1	91
BAC-2	AB010852	str. HTB139	86	EF197988	*Pseudomonas* sp. Z816X1	99
BAC-3	Ps.stutze4	*Pseudomonas stutzeri*	85	EF408245.1	*Pseudomonas fluorescens*	98
BAC-4	AB010860	str. HTB019	72	EU037340.2	Uncultured bacterium clone G3DCM-151	98
BAC-5	Y07584	clone DA026	61	EJ544338.1	*Bacillus boroniphilus* strain 822	97
BAC-6	U85871	*Halomonas variabilis*	66	FM958468.1	Uncultured γ-proteobacterium clone HG135	94
BAC-7	E.colirnE2	*Escherichia coli*	88	EU133499.1	Uncultured bacterium clone FFCH8793	99

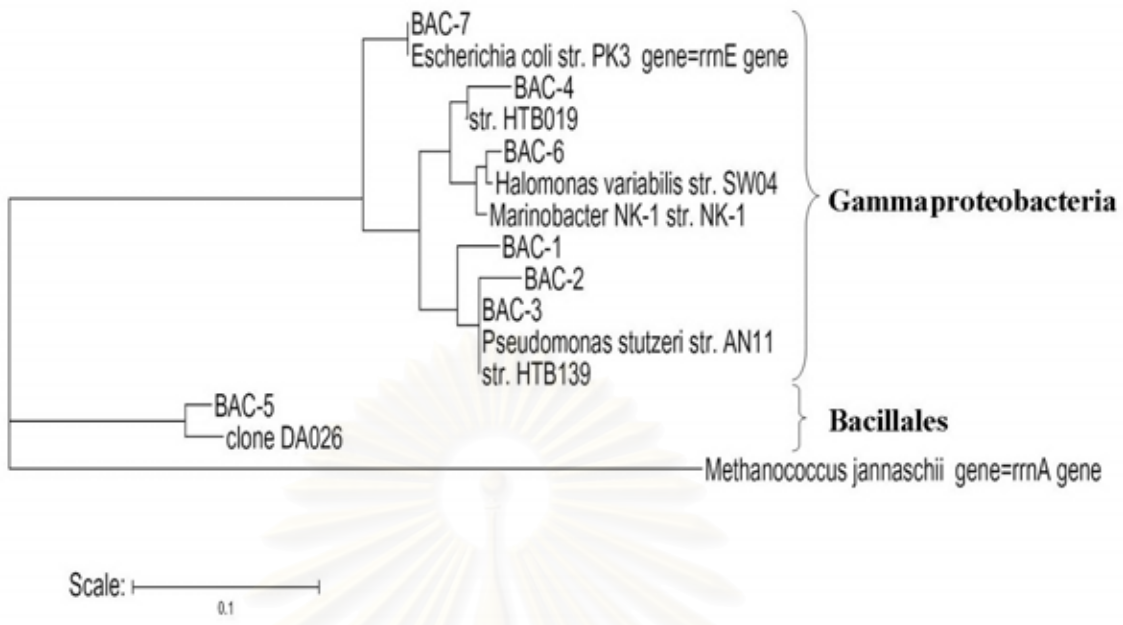


Figure 4.9 The phylogenetic tree demonstrates the relationship of seven interesting DNA band sequences (BAC-1 through 7) from DGGE gel (Figure 4.8). The tree was constructed using maximum likelihood criteria and the neighbour-joining method (RDP II version 8.1 software online packages with its database). *Methanococcus jannaschi* was used as an out-group. The bar represents 0.1 nucleotide substitution per position.

4.3.3 Effect of dissolved oxygen on inorganic nitrogen conversion and bacterial diversity in pond bottom soil from shrimp pond

There were two groups of sediment chambers in this experiment. The control chamber was set up with low DO by gently air bubble while treatment chamber was supplied with full aeration that brought DO to saturation concentration. It was found that average DO in a control chamber was 2.28 mg/L while in a treatment chamber was 7.08 mg/L and DO concentration in all chambers was nearly constant throughout 35 days of the experimental period. Other parameter including temperature, ORP, pH and alkalinity during the experiment are shown in Table 4.5.

Table 4.5 Means±SD of physical parameters and sediment quality in all experimental units during 28 day incubation.

Parameter	**Control chamber (<2.5 mg/L)**	**Treatment chamber (>7 mg/L)**
DO (mg-O_2/L)	2.28±0.44	7.08±0.46
ORP in water (mV)	63.48±18.78	72.13±22.44
ORP in sediment (mV)	-212±49.99	-185.05±56.65
Temperature in water (^oC)	25.14±0.97	25.26±0.46
pH in water	6.83±0.00, 7.42±0.03 (day 0, day 28)	7.01±0.08, 7.97±0.00 (day 0, day 28)
Total alkalinity (mg/L)	120±0.00, 70±0.00 (day 0, day 28)	120±0.00, 70±0.00 (day 20, day 28)

4.3.3.1 Water and sediment quality

Changes in inorganic nitrogen in water over experimental period were illustrated in Figure 4.10A-C. Release of ammonia was appeared after filling the seawater (Figure 4.10A) in the sediment chamber. In control chamber supplied with low dissolved oxygen (less than 2.5 mg/L), ammonia concentration was rose to the highest concentration of 4.39±0.86 mg-N/L at day 5. While, in treatment chamber with saturated dissolved oxygen (7 mg/L), ammonia concentration was lower than 3 mg-N/L. Thereafter, decrease of ammonia was found in both chambers. Ammonia concentration was below 0.1 mg-N/L after 6 days in control and 8 days in treatment chamber respectively (Figure 4.10B). A peak of nitrite was found during days 2-20 while nitrate was almost constant between day 5 and day 24 and declined afterward (Figure 4.10C).

In addition, an attempt to stimulate denitrification activity in sediment layer was performed in day 28 by adding methanol into the sediment chambers. It was found that a peak of ammonia was found in both control and treatment chambers immediately after methanol addition. On the other hand, nitrite was remain constant (Figure 4.10A-B) and reduction of nitrate was occurred after methanol addition (Figure 4.10C). During day 28 to day 35, nitrate in control chamber decreased from 2.15 to 1.16 mg-N/L and nitrate in treatment chamber decreased from 2.81 to 1.24 mg-N/L. In addition, total nitrogen and organic matter in sediment is shown in Figure 4.11-12. Total nitrogen in sediment was slightly decreased at day 35 while organic matter in the sediment was increase to 5.0% and 3.6% in control and treatment chamber respectively.

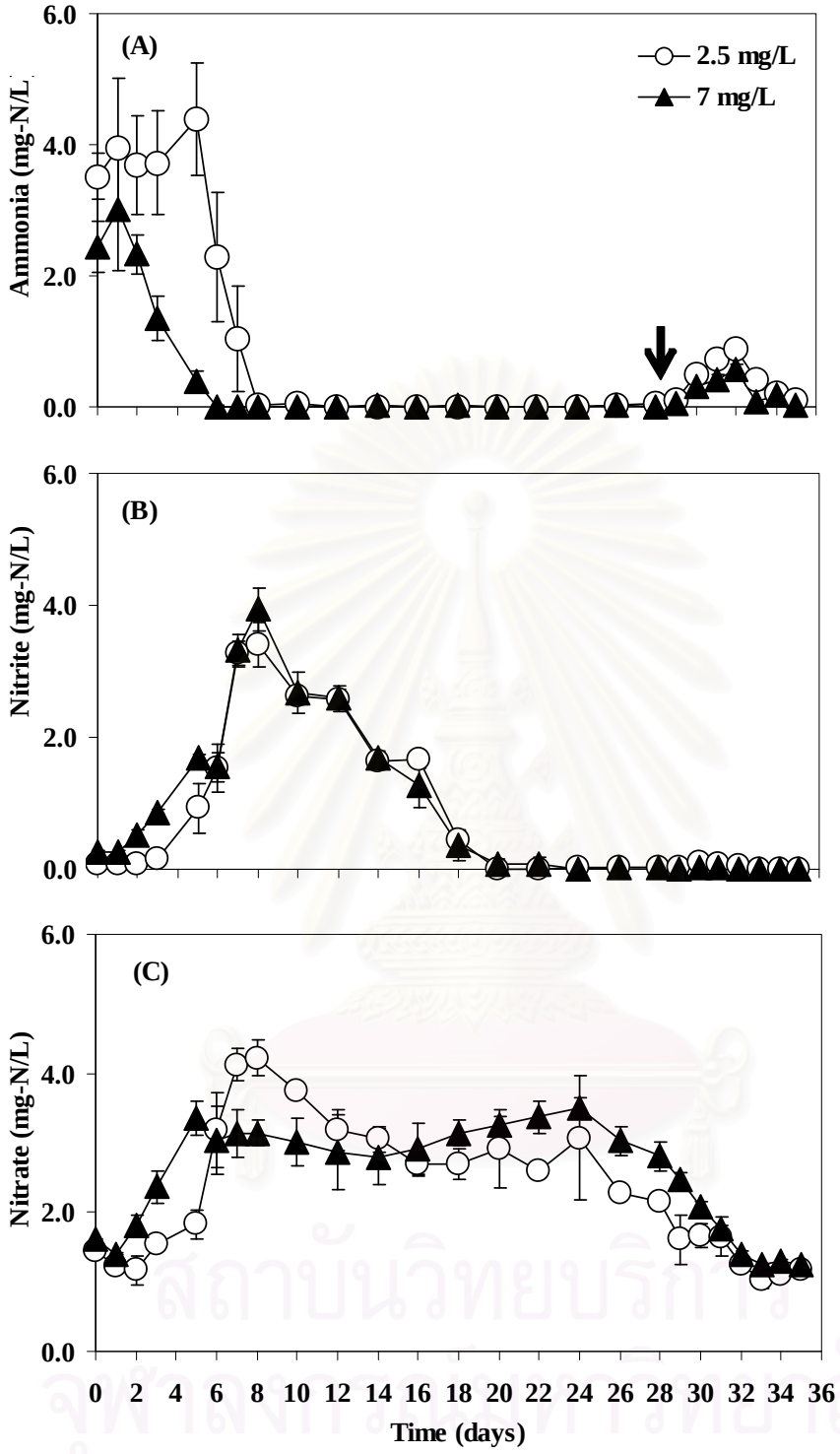


Figure 4.10 Changes of ammonia (A), nitrite (B) and nitrate (C) in water from control and treatment chambers during 35 day incubation. Noted that methanol was added to both chambers after day 28.

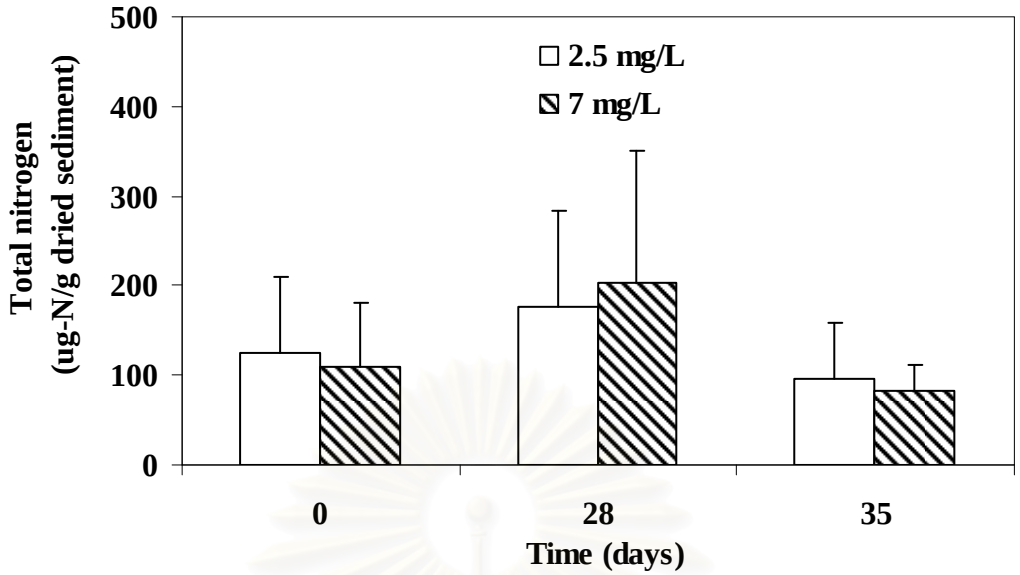


Figure 4.11 Change in total nitrogen in sediment during 35 days of incubation.

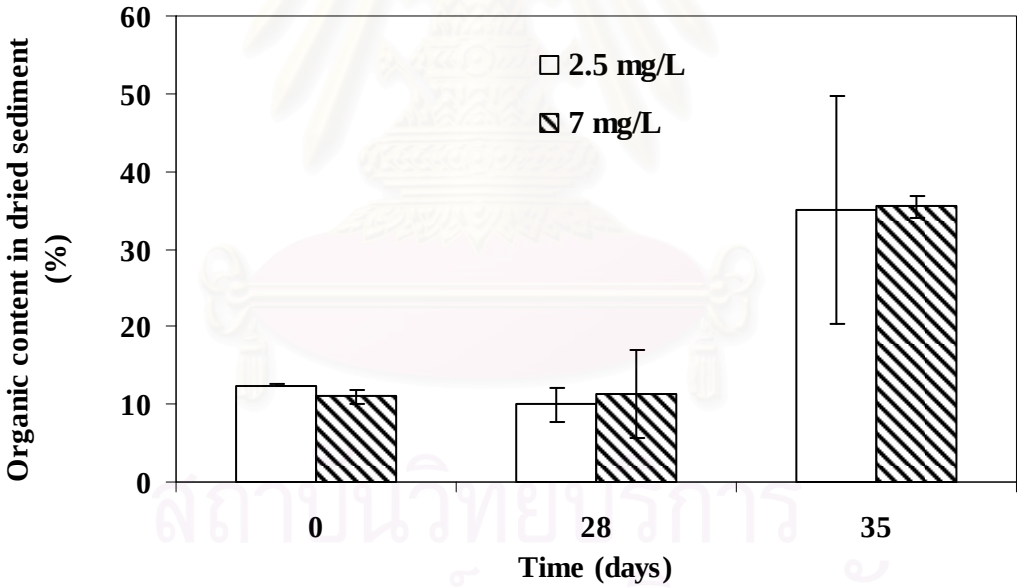


Figure 4.12 Change in organic matter in sediment during 35 days of incubation.

4.3.3.3 Bacterial diversity

Bacterial community in sediment from shrimp pond was revealed in Figure 4.13A-B. Twelve dominant 16S rDNA bands were detected in the beginning day. After incubated for 28 day, decrease of dominant DNA bands was observed. In control chamber with low oxygen, there were ten DNA bands with low intensity (Figure 4.13A). Similarity of DGGE profile of sediment bacteria at the initial day was decreased from 100% to 89% in treatment chamber while it was decreased to 53% in control chamber. However, after adding methanol, bacterial community in control and in treatment chamber was almost similar with 90% similarity (Figure 4.13B).

Comparison of bacterial diversity in control and in treatment chambers shows that trend of species richness (*R*) in control chamber was slightly decreased after incubated for 28 days and it highly decreased between day 28 to day 35 after methanol addition while constancy of species evenness (*E*) and the Shannon-Weaver index (*H*) was observe (Figure 4.14A). Likewise, species evenness and the Shannon index in treatment chamber were almost constant. However, increase in species richness was detected after methanol addition (Figure 4.14B).

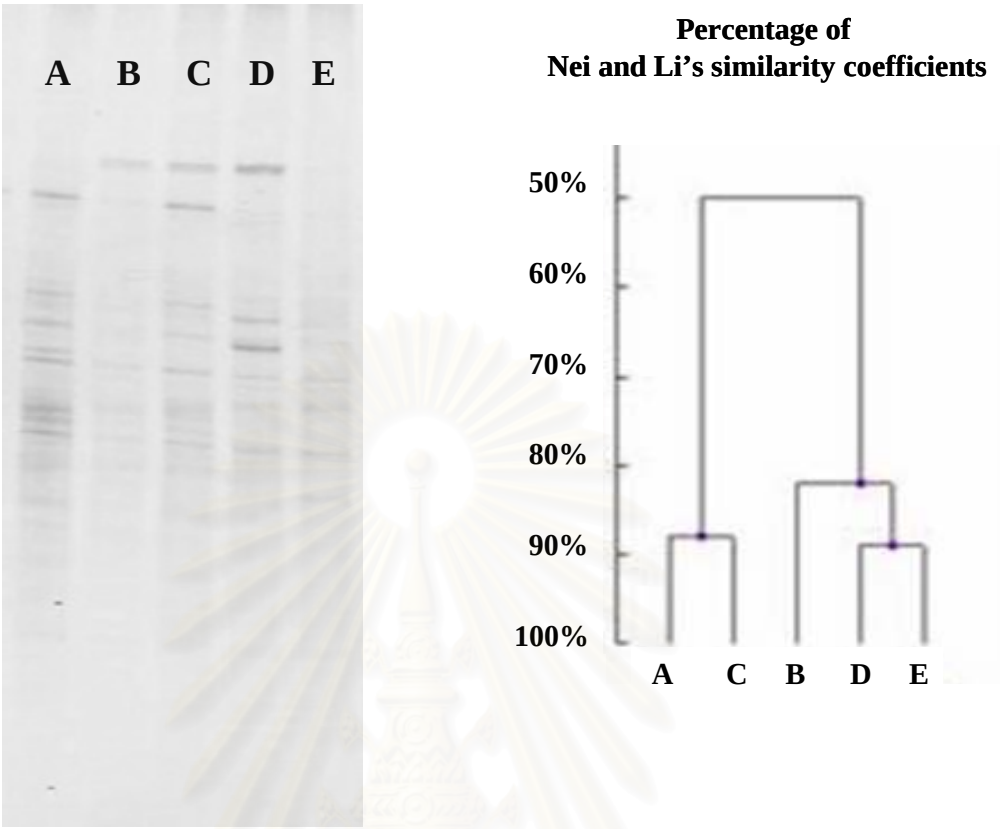


Figure 4.13 16S rDNA-based denaturing gradient gel electrophoresis (DGGE) profiles (Left) with cluster analysis (Right) of the bacterial community in sediment chambers**.** Lane A: sediment sample at the initial day; B: sediment sample from control chamber (low DO) at day 28: C: sediment sample from treatment chamber (high DO) at day 28; D: sediment sample from control chamber at day 35; and E: sediment sample from treatment chamber at day 35. Noted that methanol was added to both chambers after day 28.

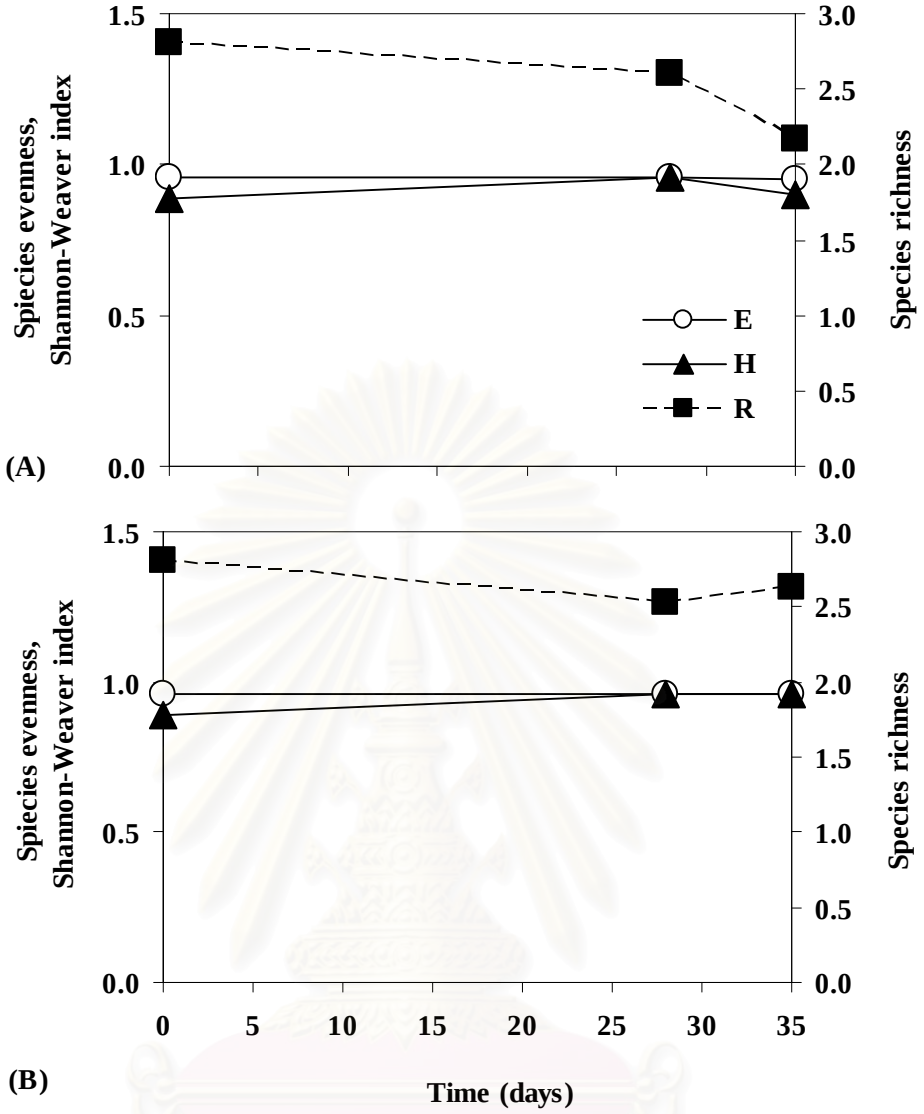


Figure 4.14 Species richness (*R*), evenness (*E*) and Shannon-Weaver index (*H*) calculated from 16S rRNA gene banding pattern amplified from sediment in control chamber supplied with 2.5 mg-O_2/L (A) and in treatment chamber supplied with 7 mg-O_2/L (B).

4.3.4 Effect of inorganic carbon addition on inorganic nitrogen conversion and bacteria diversity in pond bottom soil from a shrimp pond

This experiment composed of control sediment chambers and treatment chambers with an addition of sodium bicarbonate at the initial day. Dissolved oxygen in all chambers was maintained at between 7.94 to 8.50 mg/L. During the experiment, slightly increase of pH in the water was found in both control and treatment chambers (Table 4.6). In control chamber, total alkalinity was constant at approximately 100 mg/L while alkalinity in treatment chamber was decreased from 130 to 100 mg/L.

Table 4.6 Physical parameters and sediment quality in all experimental units throughout the experimental period.

Experimental unit	Parameter	Day 0	Day 7	Day 14	Day 29
Control chamber	pH in water	8.24	8.29	8.23	8.41
	Total alkalinity in water (mg/L)	100	100	120	100
	Water temperature (°C)	24.77	25.7	25.9	25.9
	DO (mg/L)	8.11	8.14	8.50	8.03
Treatment chamber	pH in water	8.16	8.43	8.45	8.48
	Total alkalinity in water (mg/L)	130	130	130	100
	Water temperature (°C)	25.3	25.2	25.4	25.5
	DO (mg/L)	8.00	8.35	8.23	7.94

4.3.4.1 Water and sediment quality

Release of ammonia from sediment into the water was observed in both control and in treatment chambers (Figure 4.15A). Peak of ammonia with the highest concentration of 1.98 mg-N/L was detected in control chamber at day 5. Meanwhile, the highest ammonia concentration in treatment chamber was 1.4 mg-N/L at day 3. Consequently, a peak of nitrite was found after ammonia decline (Figure 4.15B) and maximum nitrite concentration of treatment chambers (1.7 mg-N/L) was slightly lower than in control chambers (1.9 mg-N/L). During nitrite oxidation, nitrate in control was rose from 0.5 to 5.4 mg-N/L while in treatment was from 0.05 to 3.05 mg-N/L. This was followed by nitrate reduction possibly by denitrification process in the sediment (Figure 4.15C).

Figure 4.16 reveals the conversion of ammonia, nitrite and nitrate in pore water and in sediment. Increase of ammonia concentration in pore water of all chambers was detected and high ammonia was found in extracted sediment (Figure 4.16A). After 15 days of incubation, ammonia was eliminated from pore water and from extracted sediment. Trend of nitrite conversion in pore water and in extracted sediment was decreased which was after day 6 of incubation (Figure 4.16B). At the final day, nitrate reduction was found in all sediment chambers (Figure 4.16C). Decrease of organic matter content in sediment was found after an incubation period of 29 days (Figure 4.17).

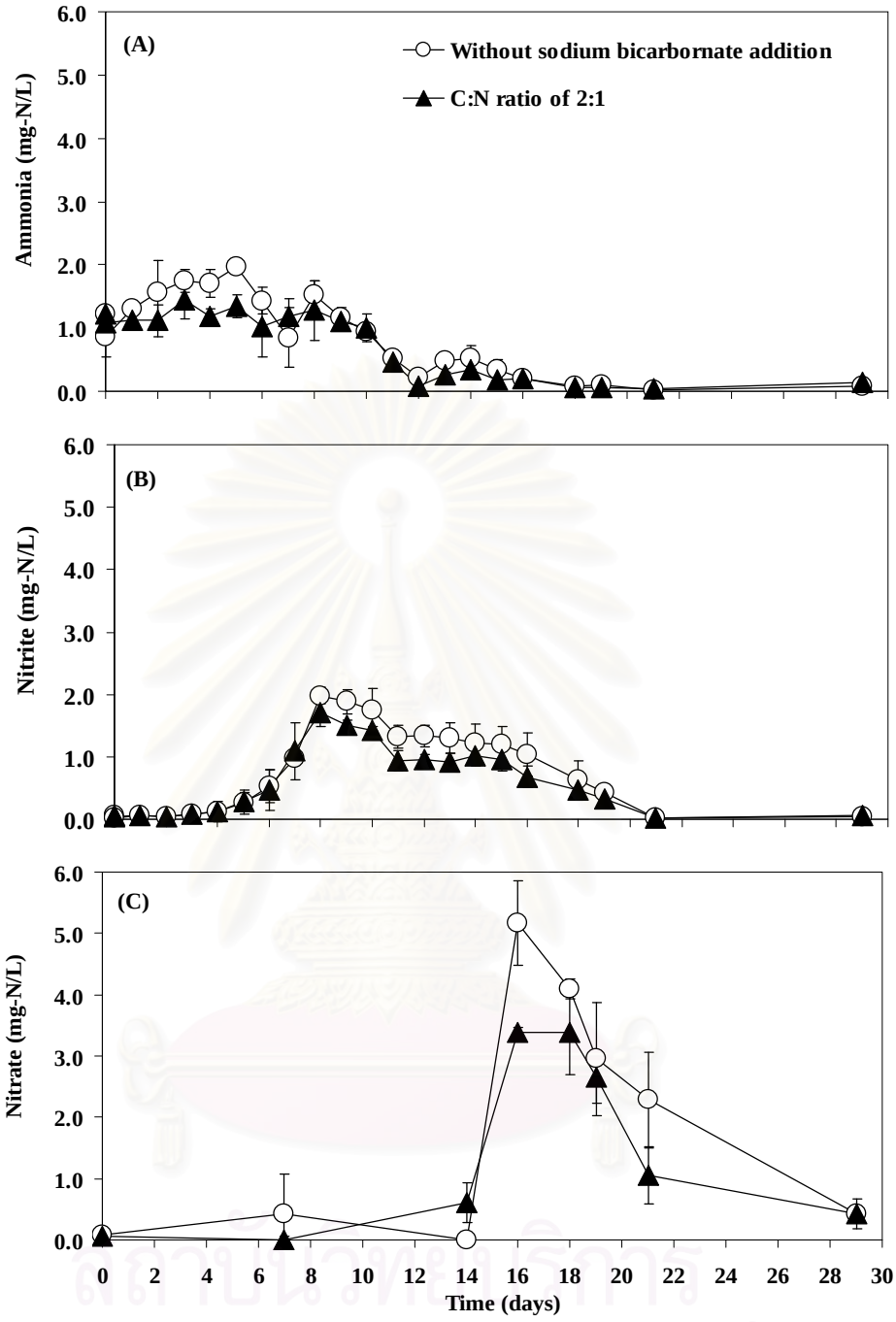


Figure 4.15 Changes of ammonia (A), nitrite (B) and nitrate (C) in water column of control (without $NaHCO_3$) and treatment chambers (with $NaHCO_3$ addition) during 29 days of experiment.

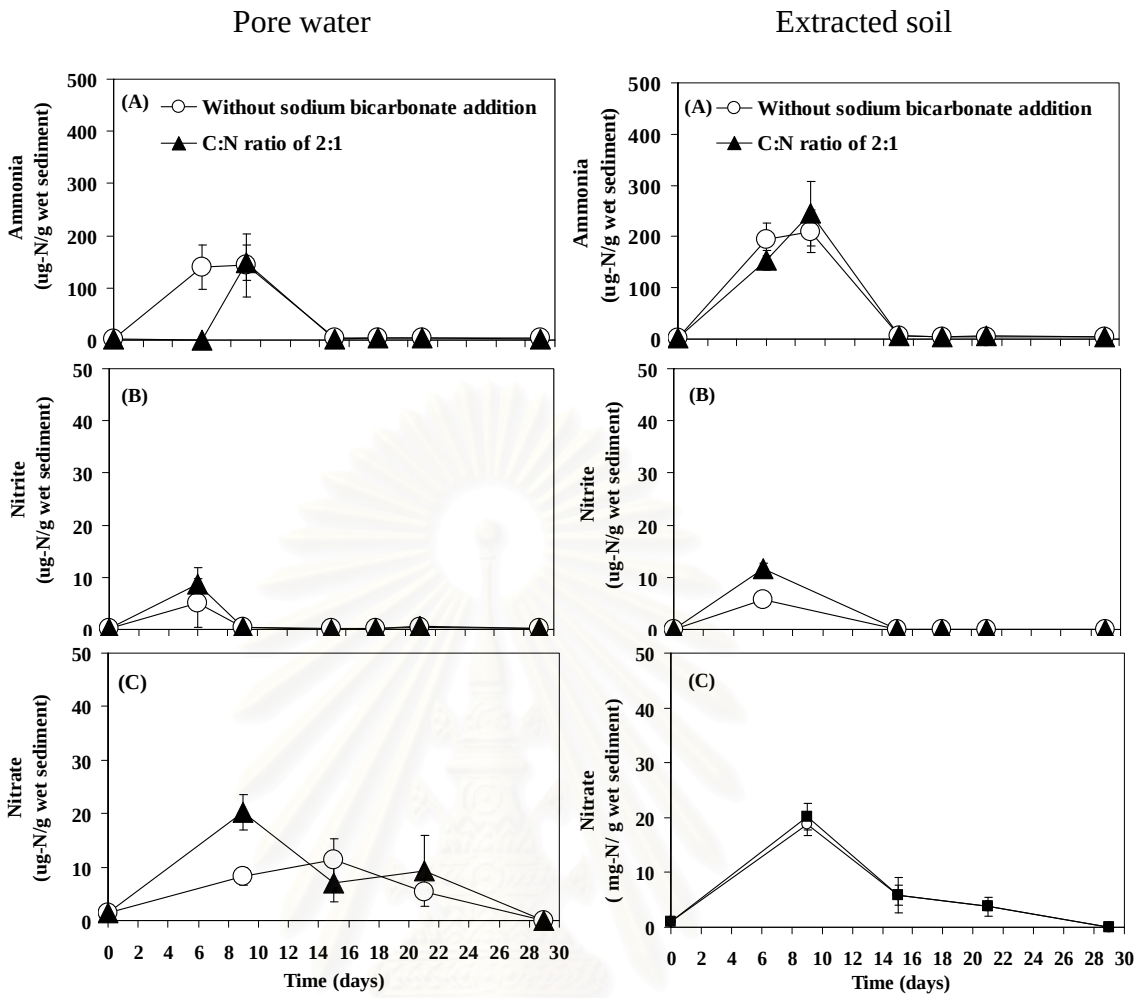


Figure 4.16 Concentration of ammonia (A), nitrite (B) and nitrate (C) in pore water and in extracted sediment of control (without $NaHCO_3$) and treatment chambers (with $NaHCO_3$ addition) during 29 days of experiment.

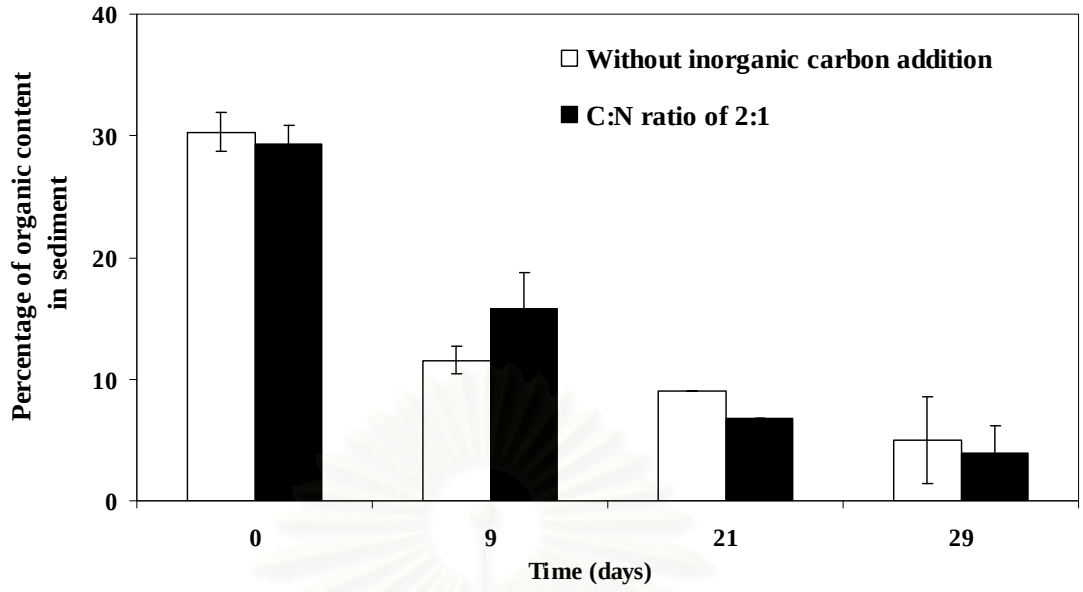


Figure 4.17 Changes in sediment organic content in control (without $NaHCO_3$) and treatment chambers (with $NaHCO_3$ addition) during 29 days of experiment.

4.3.4.2 Bacterial diversity

As shown in Figure 4.18, bacterial diversity in sediment was distinctly changed with 60% similarity after 29 days of incubation. Six dominant 16S rDNA bands were observed at the initial day. At the final day, 12 and 9 major bands were found in control and treatment chambers respectively. The similarity of bacteria in control and treatment at day 29 was 88%. With DGGE banding pattern in Figure 4.18, all diversity indices including species richness, evenness and the Shannon-Weaver was increased after 29 days of incubation (Table 4.7).

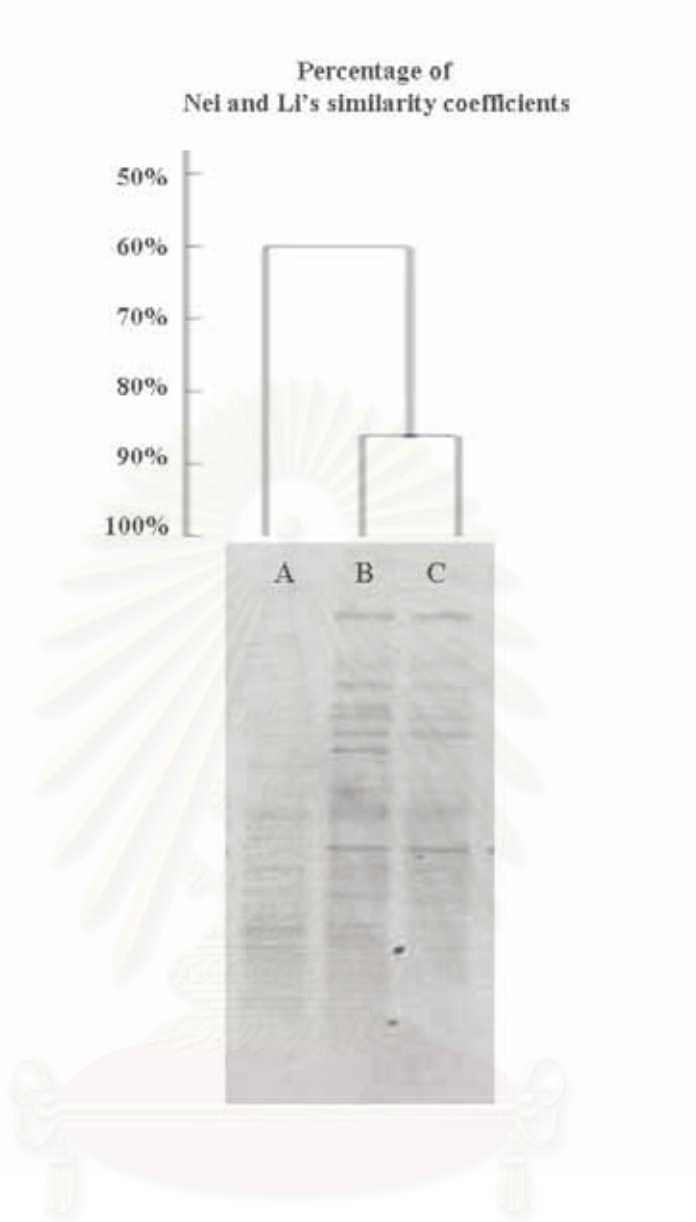


Figure 4.18 DGGE profiles with cluster analysis of the bacterial community in sediment chambers**.** Lane A: sediment sample at the beginning day; B: sediment sample from control chamber (without $NaHCO_3$) at day 29; and C: sediment sample from treatment chamber (with $NaHCO_3$) at day 29.

Table 4.7 Bacterial diversity in sediment chambers calculated from DGGE patterns (Figure 4.18).

Experimental unit	Species richness (*R*)	Species evenness (*E*)	Shannon-Weaver diversity index (*H*)
Sediment at the initial day	1.69	0.89	0.69
Sediment from control chamber at day 29 (without $NaHCO_3$ addition)	2.50	0.96	0.92
Sediment from treatment chamber at day 29 (with $NaHCO_3$ at C:N ratio of 2:1)	2.46	0.98	0.94

4.3.5 Effect of organic carbon addition on inorganic nitrogen conversion and bacteria diversity in pond bottom soil from shrimp pond

This study involved an addition of organic carbon (methanol) to enhance denitrification process in sediment chambers. With this experiment, DO in water column of all experimental units was almost constant at 6.5±1.8 mg-O_2/L throughout 48 days of the experiment due to continuous aeration. ORP values in the sediment layer were between -265 to -364 mV.

4.3.5.1 Water and sediment quality

Change in inorganic nitrogen compounds of the water column is shown in Figure 4.19. It revealed that ammonia in control chambers (without methanol) between initial day to day 9 gradually released to the highest concentration at 8.74±2.16 mg-N/L (Figure 4.19A) and then decreased to 0.29±0.05 mg-N/L at day 18. For treatment-1, after adding methanol with C:N ratio 2:1, ammonia was continuously released to the highest concentration (9.73±2.17 mg-N/L) at day 9; then it was removed from the water in day 27. The similar result was found in treatment-2 that supplemented with higher methanol (C:N ratio at 4:1) (Figure 4.19B). Peak of

nitrite was found in all experimental units as following ammonia oxidation. At day 46, all nitrite was eliminated from the chambers.

Ammonia concentration in pore water of all experimental units was high at the initial day and it then continuously decreased to lower than 15 mg-N/g wet sediment (Figure 4.20). During 48 day incubation, concentrations of nitrite in both pore water and extracted sediment of all experimental units were constantly low concentration (Figure 4.20A-B). Decrease of nitrate in sediment was found in all sediment chambers after incubating for 48 days (Figure 4.20C). Regarding to sediment analysis, the result showed that organic content and total nitrogen in sediment was constant in all experimental units (Figure 4.21, 4.22).

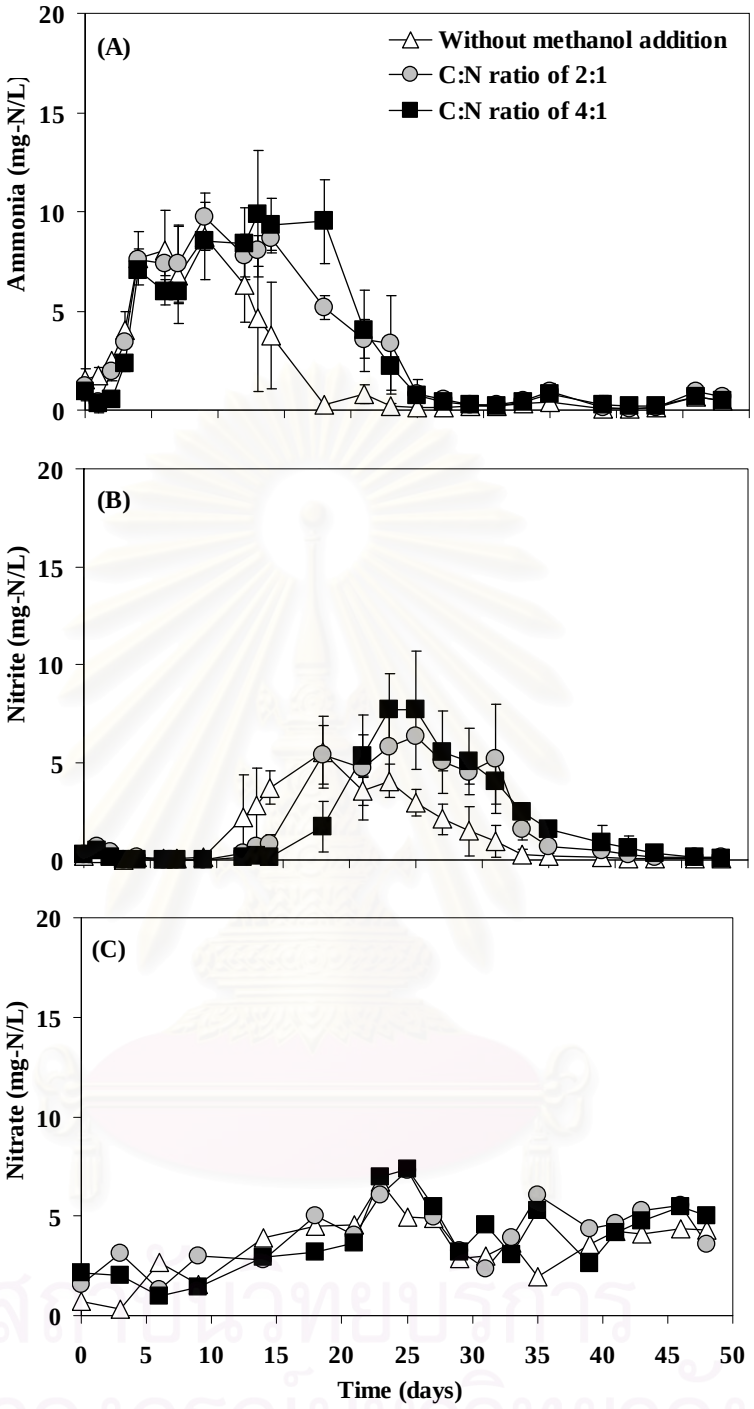


Figure 4.19 Changes in ammonia (A), nitrite (B) and nitrate (C) in water column of the sediment chambers of control (without methanol), treatment-1 (with methanol at C:N = 2:1) and treatment-2 (with methanol at C:N = 4:1) during 48 day incubation.

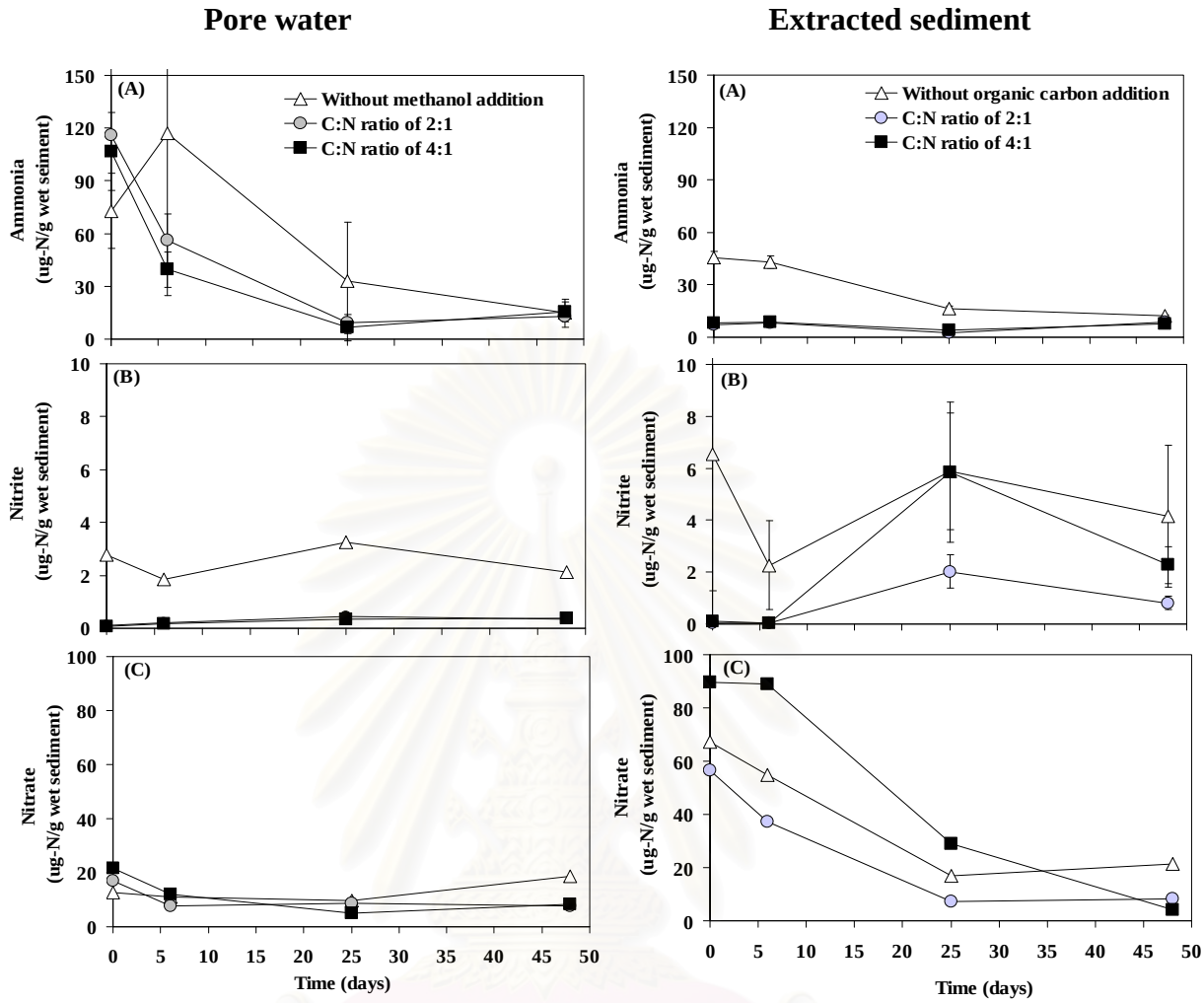


Figure 4.20 Concentrations of ammonia (A), nitrite (B) and nitrate (C) in pore water and extracted sediment from sediment chambers of control (without methanol), treatment-1 (with methanol at C:N = 2:1) and treatment-2 (with methanol at C:N = 4:1) during 48 day incubation.

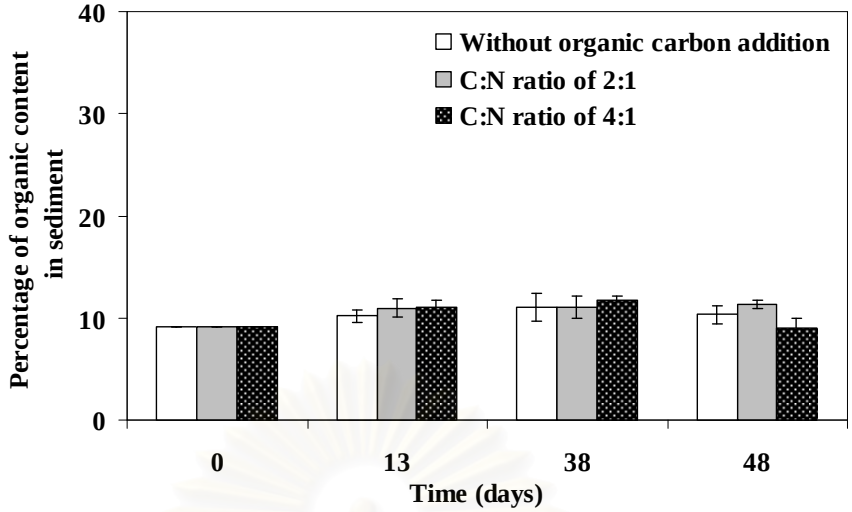


Figure 4.21 Changes of sediment organic content from sediment chambers of control (without methanol), treatment-1 (with methanol at C:N = 2:1) and treatment-2 (with methanol at C:N = 4:1) during 48 day incubation.

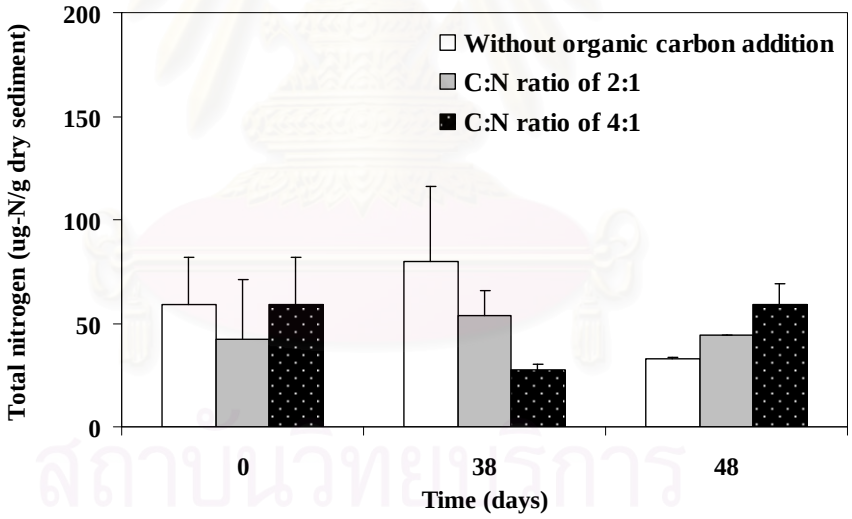


Figure 4.22 Change in total oxidized nitrogen of the sediment chambers of control (without methanol), treatment-1 (with methanol at C:N = 2:1) and treatment-2 (with methanol at C:N = 4:1) during 48 day incubation.

4.3.5.2 Bacterial diversity

With the duplicate of DGGE analysis for bacterial diversity, DGGE profile of 16S rRNA gene (Figure 4.23) showed that bacterial community in the sediment was distinctly changed after 48 day of the experimental period. At least 29 major bacterial species indicated by intense DGGE bands were detected at the initial day. After incubation for 48 days, at least 4 new bacteria band were found while at least 6 species were disappeared. At day 48, different DNA banding patterns and intensity were observed in control and treatment chambers.

To analyze the structure of sediment bacterial community based on the DGGE patterns of the 16S rDNA fragments, the Shannon-Weaver index and a cluster analysis was applied. It was found that, after 48 days incubation, the Shannon-Weaver index and species richness was highest in control chamber. Although a slightly decrease of diversity index was observed in all sediment chambers which incubated for 48 days. The diversity index was lowest in treatment-2 which had high organic carbon addition (Table 4.8).

Cluster analysis of the DGGE patterns with UPGMA method is illustrated in Figure 4.24. It was found that three major clusters were generated in which bacterial community in sediment chamber strongly differed after 48 days of incubation. In control chamber, similarity of bacterial community was decreased from 100% at day 0 to 71% at day 48. Bacterial diversity of treatment-1 and 2 was rather similar with 94% similarity and the similarity between initial day and day 48 was 71%.

To identify the dominant bacteria in the sediment chamber, a total of 16 interested 16S rDNA bands were excised from the gel (Figure 4.24), re-amplified and sequenced. The closest relatives were determined by DNA sequence comparison with the RDP II and NCBI databases (Table 4.9). It was found that DNA bands namely ORG-10, ORG-12 and ORG-15 were found in all sediment chambers during the incubation period. With RDP II database, these DNA bands were closely related to *Thermobispora bispora, Pseudomonas* sp. and Clone env. OPS17, respectively. While in NCBI database, these were identifiec as uncultured actinobacterium, uncultured bacterium and uncultured *Salegentibacter* sp., respectively. In Figure 4.24, DNA bands ORG-1, OGR-2, ORG-11, ORG-14 and

ORG-15 were found only in the initial day. Succession of new bacterial species indicated by DNA bands of ORG-3, ORG-4, ORG-6, ORG-7, ORG-8 and ORG-9 was found in day 48 of treatment chambers.

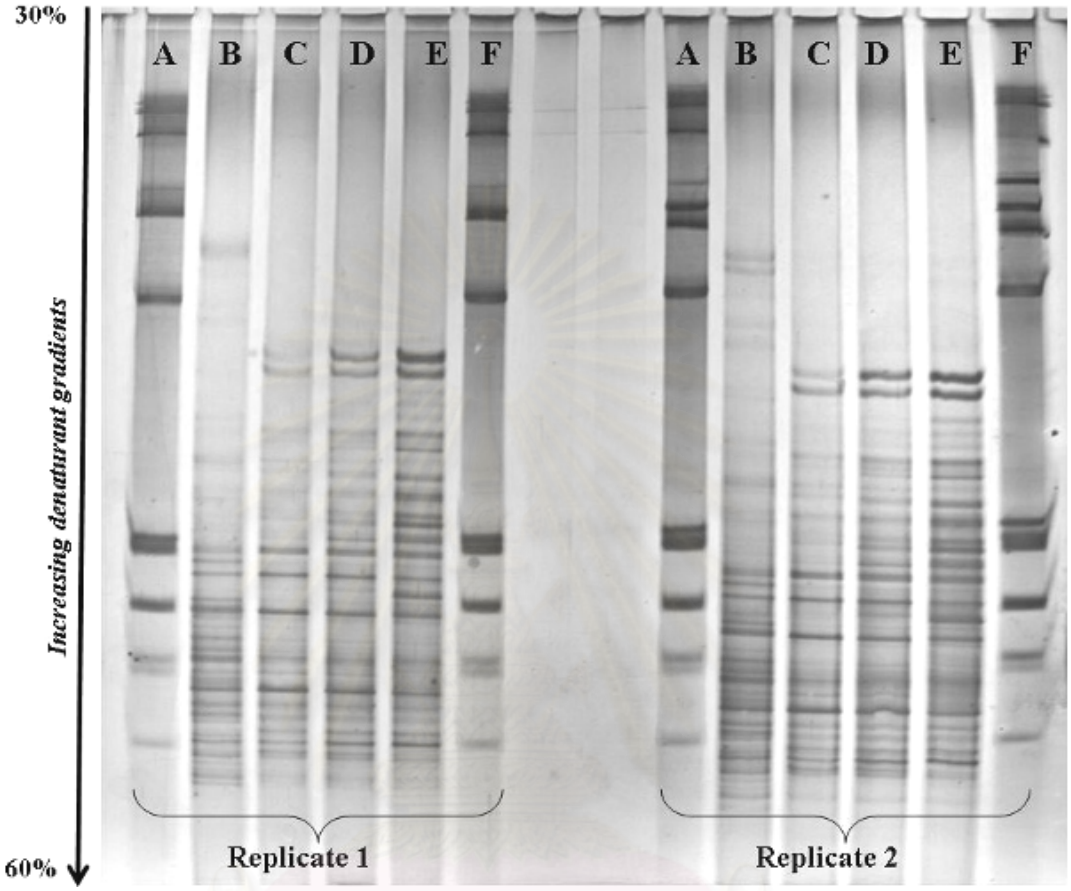


Figure 4.23 DGGE profiles of bacteria in the sediment at the initial day and after 48 days incubation. 16S rRNA gene was amplified with primers 338f plus GC clamp and 518r. The gel was performed with duplicate. Lanes A and F, DNA ladder (ϕX174 DNA-HaeIII digest); lanes B, sediment sample at day 0; lanes C, sediment from control (without organic carbon addition) after 48 days of incubation; lanes D, sediment from treatment-1 (C:N ratio of 2:1) after 48 days of incubation; lanes E, sediment from treatment-2 (C:N ratio of 4:1) after 48 days of incubation.

Table 4.8 Bacterial diversity in sediment chambers calculated from DGGE patterns.

Experimental unit	**Species richness (*R*)**	**Species evenness (*E*)**	**Shannon-Weaver diversity index (*H*)**
Sediment at day 0	15.13	0.97	1.43
Sediment from control at 48 day incubation (without methanol addition)	15.42	0.96	1.40
Sediment from treatment-1 at 48 day incubation (C:N ratio of 2:1)	14.03	0.94	1.38
Sediment from treatment-2 at 48 day incubation (C:N ratio of 4:1)	14.66	0.93	1.38

Moreover, intensity of DNA bands especially ORG-5 was found highest in treatment-2 chambers with high methanol supplement (C:N = 4:1). Identification of this band showed that it could possibly belong to *Thioalcalovibrio denitrificans* of the Gammaproteobacteria. In order to obtain more information of sediment bacterial groups, a phylogenetic distance tree for DNA bands ORG-1 to ORG-16 was analyzed and the results are shown in Figure 4.25. Six lineages of bacterial diversity were found in sediment samples including phylum Proteobacteria, Firmicutes, Actinomycetales, Unclassified bacteria, Chloroflexi and Bacteriodetes. DNA bands ORG-3, ORG-5, ORG-7, ORG-8, ORG-2 and ORG-12 were grouped into Proteobacteria while all of them were most closely related to class γ-proteobacteria.

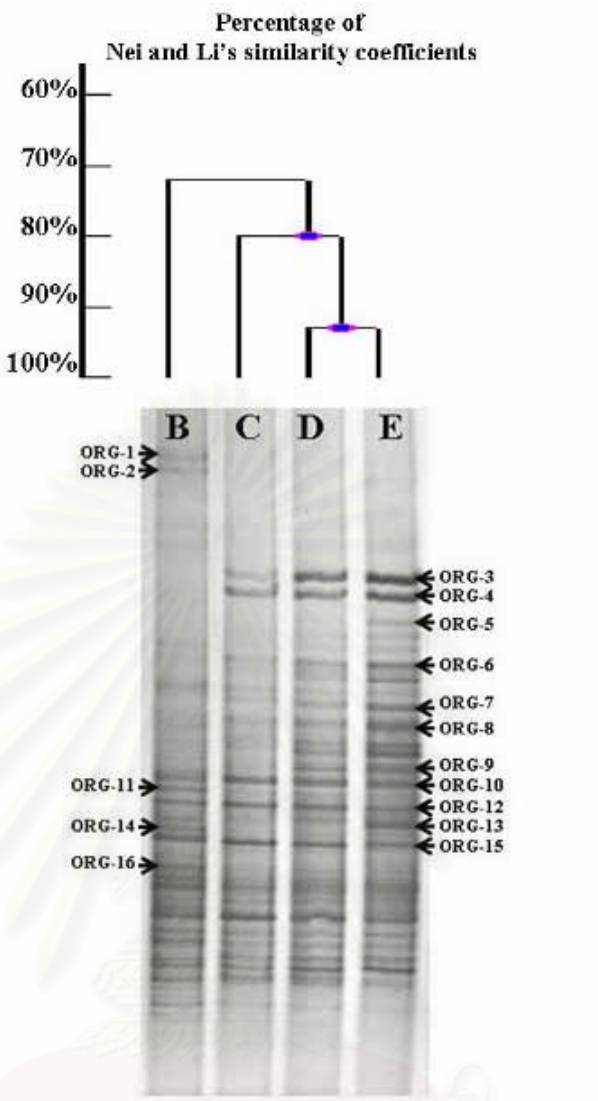


Figure 4.24 16S rDNA-based denaturing gradient gel electrophoresis (DGGE) profiles with cluster analysis of the sediment bacterial community in sediment chambers. The terms B denoted sediment sample at day 0; C, sediment sample from control (without methanol addition) after 48 days of incubation; D, sediment sample from treatment-1 (C:N ratio of 2:1) after 48 days of incubation; E, sediment sample from treatment-2 (C:N ratio of 4:1) after 48 days of incubation. Arrows indicated selected DNA band for further sequencing.

Table 4.9 Identification of DNA sequences of selected 16S rDNA bands using RDP II and NCBI databases.

DNA band	RDP II		NCBI	
	ID	**Description**	**ID**	**Description**
ORG-1	AF029049	Clone SB-34	DQ137920	Uncultured eubacterium clone U27-1
ORG-2	Str. Thd2.	str. Thd2.	EU542478	Uncultured bacterium clone Er-MLAYS-58
ORG-3	V.nereis	*Vibrio nereis* CIP103194	AM884367	*Vibrio* sp. NA21
ORG-4	C.purnoly	*Clostridiun purinolyticum*	EU246261	Uncultured organism clone MAT-CR-P6-A01
ORG-5	U77480	Unnamed organism	AF126545	*Thioalcalovibrio denitrificans*
ORG-6	AF044946	*Peptostreptococcus* S1 str. S1	EU246262	Uncultured organism clone MAT-CR-P6-A03
ORG-7	AF026985	Clone OPB37	AB426189	Uncultured bacterium clone mbI-b7.
ORG-8	U65012	*Pseudomonas stutzeri* str. Zobell ATCC 14405	EU814518	*Pseudomonas* sp. th16
ORG-9	Plc.citreu	*Planocuccus citreus* NCIMB 1493	DQ861235	*Planococcus* sp. PB28 1
ORG-10	Ths.bispo	*Thermobispora bispora* str. R51 ATCC 1999	AJ575538	Uncultured actinobacterium clone D2
ORG-11	Stm.thrulg	*Streptomyces thermovulgaris*	DQ909369	Uncultured bacterium clone FS396_454_1000bp_0470B
ORG-12	AB021318	*Pseudomonas* MBIC3963	EU700188	Uncultured bacterium clone STU46
ORG-13	AB015536	str. BD2-6	EU043588	Uncultured *Nitrospira* sp. clone GASP-0KA-518-F05
ORG-14	AF029049	Clone SB-34	DQ463225	Uncultured bacterium clone DS3-46
ORG-15	AF170749	str. QSSC9-3	EU328085	Uncultured *Salegentibacter* sp. clone Y20
ORG-16	AF018199	Clone env. OPS17	EU645027	Uncultured bacterium clone Moo60

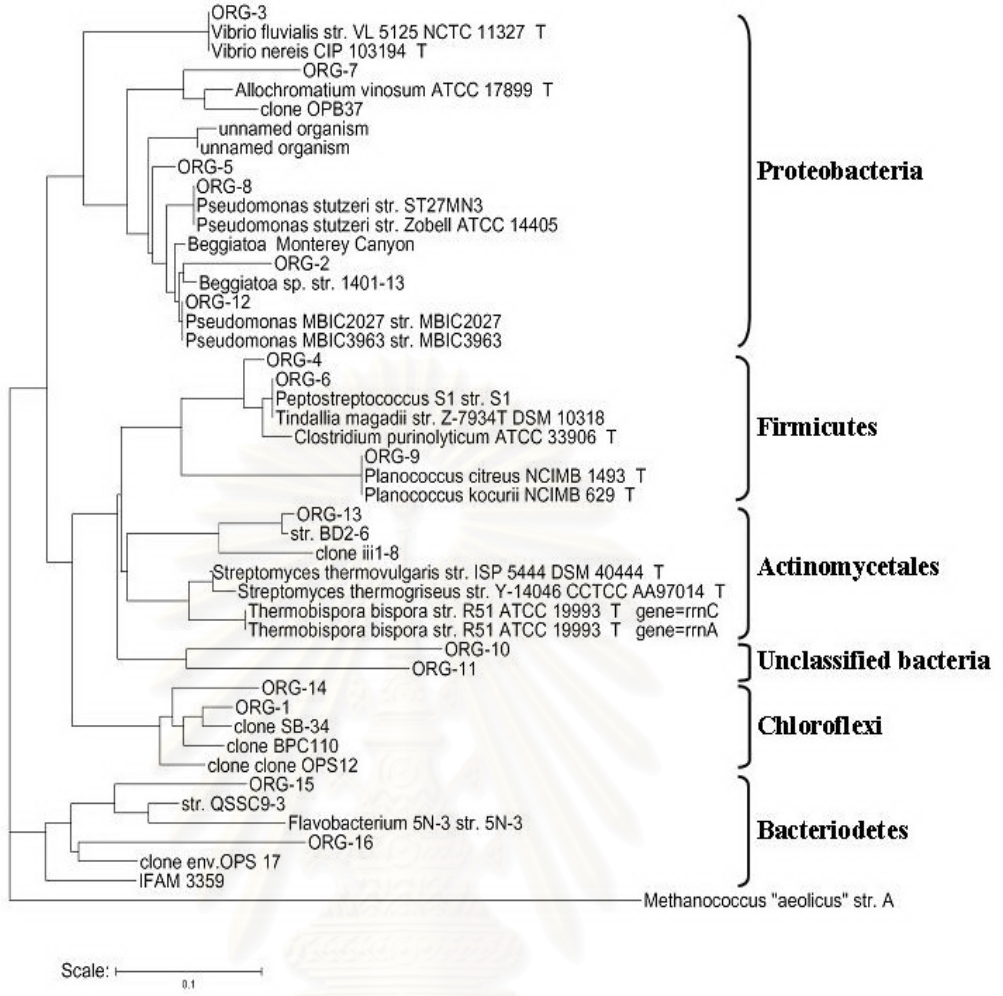


Figure 4.25 Phylogenetic tree generated from the alignment of partial 16S rRNA gene sequences from sediment bacterial. DGGE bands analyzed in this study are ORG-1 to ORG-16 (Figure 4.24). The scale bar corresponds to 10 nucleotide substitution per100 nucleotide position

4.3.6 Effect of illumination on inorganic nitrogen conversion of sediment from shrimp pond under laboratory condition

The sediment chambers in this experiment were divided into light and dark chambers. The light chamber was illuminated at 1000 lux while dark chamber was covered with black plastic sheet. Several parameters including water temperature, alkalinity, ORP in water and in sediment, and DO was measured at day 0, 26, 32 and 46 and the data are shown in Table 4.10. Water temperature was similar in all chambers while fluctuation of total alkalinity was detected between 110 to 200 mg/L in light chamber and between 110 to 150 mg/L in dark chamber. ORP value in water ranged between 228.9 to 315.1 mV in light chamber and 227.1 to 308.5 mV in dark chamber. In sediment, average ORP in light chamber was -146.3±12.38 mV while in dark chamber was -150±19.20 mV.

Table 4.10 Physical parameters in the experimental chambers throughout 46 days of experiment.

Experimental unit	Parameter	Day 0	Day 26	Day 32	Day 46
Light chamber	Water temperature (ºC)	27.8	26.8	27.1	26.7
	Alkalinity (mg/L)	120	110	200	200
	ORP in water (mV)	257.5	228.9	315.1	311.3
	ORP in sediment (mV)	-142.2	-130.6	-155.8	-156.6
	DO (mg/L)	6.30	8.30	6.55	8.77
Dark chamber	Water temperature (ºC)	27.9	26.8	27.3	26.8
	Alkalinity (mg/L)	120	110	120	150
	ORP in water (mV)	247.6	227.1	308.5	300.0
	ORP in sediment (mV)	-126.9	-163.8	-142.8	-168.2
	DO (mg/L)	6.21	5.53	6.37	7.36

4.3.6.1 Water quality

During 46 days incubation period, changes in inorganic nitrogen compounds and chlorophyll-*a* are shown in Figure 4.26. At the initial day to day 4, it was found that ammonia was gradually released to water column (Figure 4.26A). It was followed by ammonia oxidation to nitrite but lower nitrite was found in light chamber (Figure 4.26B). The highest nitrate concentration was found in day 3 and it was then reduced to lower than 4 mg-N/L in both light and dark conditions. To simulate nitrogen loading into the system, 0.33 g shrimp feed pellets was added in both experimental chambers in day 16 in order to provide 2 mg-N/L organic nitrogen loading. The results revealed that small peak of ammonia and nitrite were found after organic nitrogen addition in day 16 but the concentrations were lower than 0.05 mg-N/L while nitrate was rather constant (Figure 4.26C).

Increase of organic nitrogen loading by an addition of 0.67 g feed pellets (or 4 mg-N/L) at day 27, resulted in an increase of chlorophyll-*a* concentration in light chamber (Figure 4.26D). With dark chamber, on the other hand, ammonia oxidation following by nitrite oxidation occurred consequently and nitrogen conversion as illustrated by lower peaks of ammonia and nitrite was visibly differ from those found in light chamber. With the highest loading of 1.67 g feed pellets (or 10 mg-N/L) at day 32, ammonia and nitrite was highly accumulated in both light and dark conditions. Higher concentrations of ammonia, nitrite and chlorophyll-*a* were found in sediment chamber incubated with light. As a result, phytoplankton bloom in light chamber was found between days 34 to 42.

4.3.6.1 Eukaryotic plankton diversity

At the final day, plankton diversity in the light chamber was analyzed by PCR-DGGE technique. DGGE profile of 18S rRNA gene amplified from eukaryotic plankton DNA is shown in Figure 4.27. It was found that at least eleven planktons with three dominant species were found in light sediment chamber.

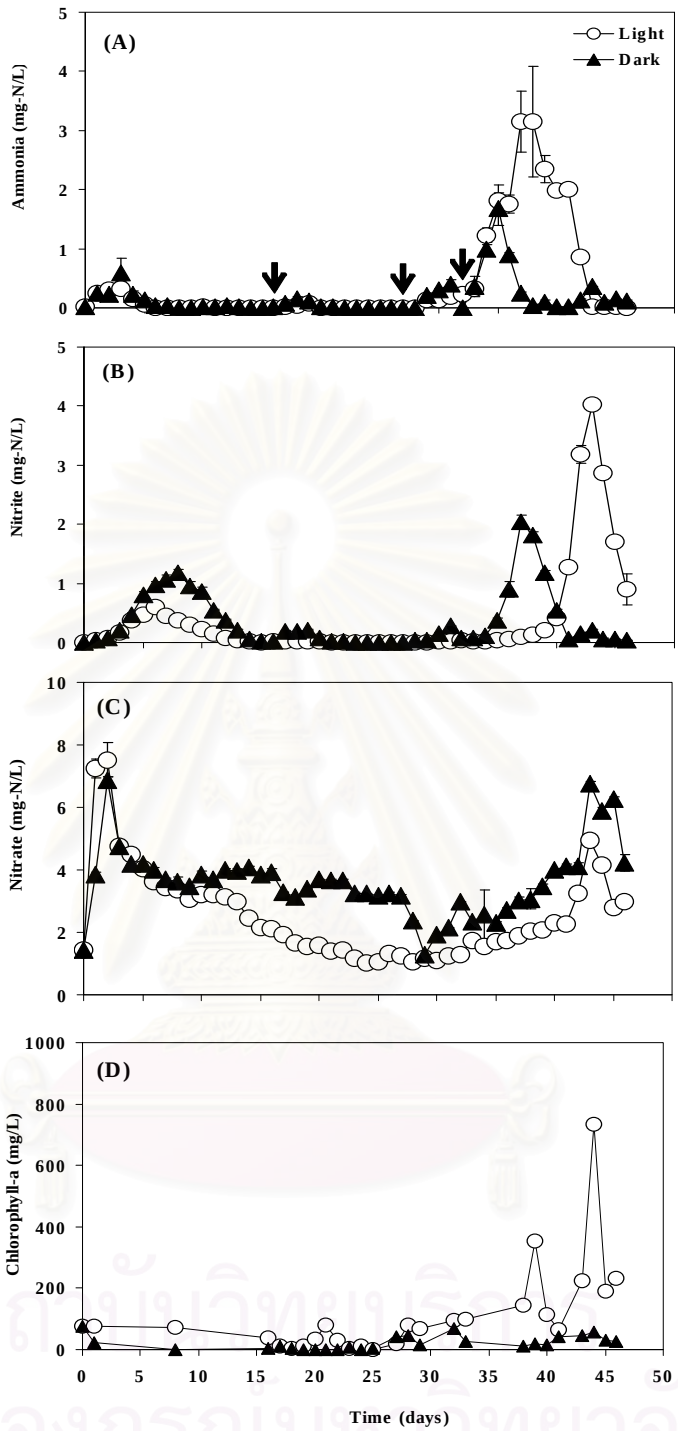


Figure 4.26 Changes in ammonia (A), nitrite (B) nitrate (C) and chlorophyll-*a* (D) during 46 days of incubation. Arrows represented the addition of shrimp feed.

Figure 4.27 DGGE band pattern of 18S rRNA gene amplified with a forward primer 1427 plus GC clamp and a reverse primer 1616. The duplicate of DGGE pattern was evaluated. Arrows indicate the dominant DNA bands.

4.4 Discussion

The experiments in this chapter consisted of five experiments that evaluating effects of environmental factors including salinity, dissolved oxygen, inorganic carbon addition, organic carbon addition, and illumination on inorganic nitrogen conversion and bacterial diversity in sediment from shrimp pond under laboratory condition.

With this study, the experiments were performed independently and sediment sample was collected only for each experiment. Hence, data such as nitrogen concentrations and removal rates from each experiment could not be directly compared. To explain the effects of each environmental factor, characteristic of nitrogen conversion was clarified and illustrated in Figure 4.28. Principally, when sediment chamber is filled with water with sufficient dissolved oxygen, ammonia will be released after the decomposition of organic nitrogen. Next, a peak of ammonia is then declined as it is converted to nitrite. Elimination of nitrite from water column is an indicator for the complete nitrification process. With this comparison, changes of nitrogen conversion characteristics were compared in percent with the control of each experiment. The ideal characteristic of the consequence nitrification and denitrification processes was that ammonia must be quickly converted to nitrite. Peaks of ammonia and nitrite must be at low concentrations. Finally, with complete denitrification process, nitrate accumulation must not be found.

The results in Table 4.11 illustrated that only an addition of HCO_3 could reduced the peak of ammonia by accelerating nitrification process while other attempts such as salinity changes, low DO, adding methanol and illumination induced high ammonia in the water column. Increase of ammonia was probably an effect of organic nitrogen decomposition or ammonification process. Addition of organic carbon (methanol) and illumination prolonged ammonia and nitrite treatment with higher peak concentrations but did not affected denitrification process since accumulation of nitrate was not found.

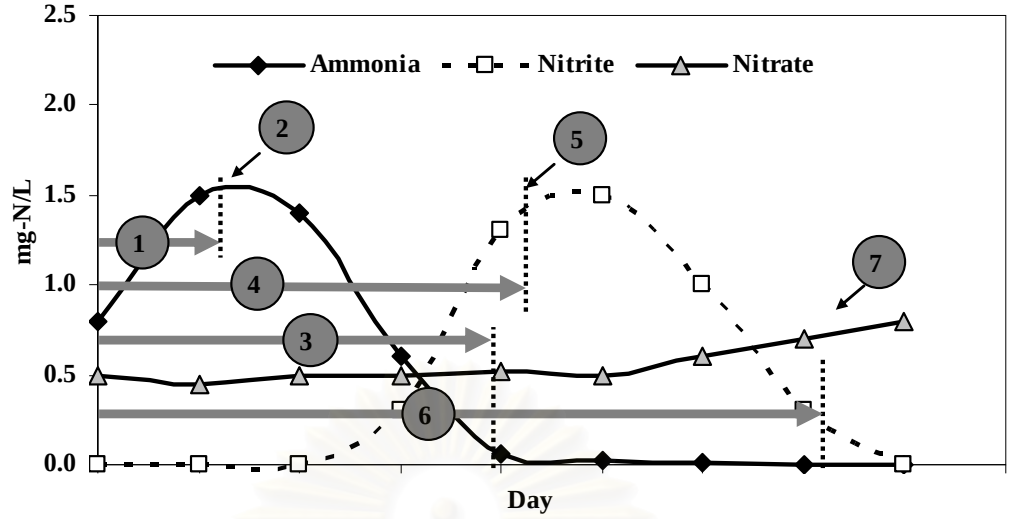


Figure 4.28 Characteristic of inorganic nitrogen conversion in the sediment chamber including: (1) time to the highest ammonia concentration, (2) peak concentration of ammonia, (3) time for total elimination of ammonia, (4) time to the highest nitrite, (5) peak concentration of nitrite, (6) time for total elimination of nitrite, and (7) accumulation of nitrate.

Table 4.11 Summary of the change of inorganic nitrogen conversion after an alteration of environmental factors. The shown data were in percent compared with control. Minus data (lower than control) are shown in bracket.

	Salinity changed from 5 to 20 PSU	**Low DO (2.5 mg/L)**	**+$NaHCO_3$**	**+methanol C:N = 2:1**	**+methanol C:N = 4:1**	**Illumination**
1. Time to max NH_3 (day)	(-14.29 %)	+400.00 %	(-40.00 %)	0.00 %	+44.44 %	+8.57 %
2. Peak NH_3 (mg-N/L)	+6.67 %	+45.36 %	(-26.90 %)	+14.59 %	+16.47 %	+96.88 %
3. Time for NH_3 elimination (day)	(-9.09 %)	(-50.00 %)	0.00 %	+61.11 %	+61.11 %	+13.16 %
4. Time to max NO_2 (day)	0.00%	0.00%	0.00%	+38.89	+27.78	+16.22
5. Peak NO_2 (mg-N/L)	(-38.57 %)	+15.54 %	(-13.64 %)	+15.43 %	+44.46 %	+96.57 %
6. Time for NO_2 elimination (day)	0.00%	0.00%	0.00%	+30.30	+39.39	ND*
7. NO_3 accumulation	Yes	Yes	No	No	No	No

***ND** = not determined

4.4.1 Effect of environmental factors on inorganic nitrogen conversion.

With this study, it was hypothesized that inorganic nitrogen treatment is the natural nitrification-denitrification processes occurred mainly at the pond bottom. Changes in environmental conditions that possibly affect bacterial activity and diversity would have accelerating or delaying the inorganic nitrogen conversion

rate. Discussion on effect of each environmental factor on nitrogen conversion was as the following:

4.4.1.1 Salinity

Sudden change of salinity from 5 to 20 PSU was the first tested factor. In general, it is known that salinity controls the communities and functions of bacteria in the environments (Abed, *et al*., 2007; Freitag and Prosser, 2003). Regarding to high salinity aquaculture in inland or freshwater areas such as black tiger shrimp or white shrimp culture in Thailand, It may result in changes in the community of natural bacteria and their activity, especially in the efficiency of nitrogen removal. Hence, there were several publications evaluated that salinity has impact on nitrification and denitrification process (Uygur 2006; Liu *et al*., 2008; Rene *et al*, 2008).

In this study, sediment from shrimp pond was packed in the control sediment chamber and filled with low salinity seawater (5 PSU). This salinity was closed to the original salinity in the shrimp pond in Pathum Thani Province. With treatment chamber, on the other hand, it was filled with high salinity seawater (20 PSU). It was found that trend of inorganic nitrogen conversion in water column of all experimental chambers was almost similar (Figure 4.3A-C). Since the production of ammonia was detected after filling seawater (Figure 4.3A), it was due to the mineralization of organic nitrogen compounds to ammonia by heterotrophic bacteria (Jones *et al*., 1995; Moriarty, 1977). As nitrite was not detected during day 0-5 of incubation, it was possible that ammonia oxidizing bacteria (AOB) was not fully growth and activated. Likewise, several studies (Hu *et al*., 2003; Kim *et al*., 2005; Michauda *et al*., 2006) reported that bacteria have the potential to adapt to the change in their environments such as pH, temperature, nutrients, DO and also salinity. In general, AOB have a significant activity after incubated for at least three weeks (Zhang *et al*., 2008). It was unlike our result that increase of nitrite was found during day 6-9 of incubation (Figure 4.3B). This was indicated that the natural AOB in sediment from shrimp pond rapidly adapts to a wide ranges of salinity (*e.g.* 5-20 PSU). Another possibility was due to high organic content in the sediment (see in Figure 4.5) could stimulate activity of heterotrophic bacteria and their metabolism hence induced the activity of autotrophic bacteria (Verhagen *et al*., 1992). Many

studies reported that some nitrifying bacteria are not strictly autothrophic condition whereas some of these can grow on heterotrophic condition (Cafferey *et al*., 2007: Jie *et al*., 2008). Despite the concentration of ammonia and nitrite in all chambers was eliminated after 16 days of incubation period and a constant of nitrate concentration was observed (Figure 4.3C). It was suggested that the rate of nitrate production by nitrification process under aerobic condition was probably equal to the rate of nitrate reduction by denitrification process under anaerobic condition. That was confirmed by the value of DO in water column that support nitrification process and low ORP (-121 to -356 mV) in sediment layer that indicated nitrate reduction by denitrification process (Figure 4.1). With this assumption, nitrate is produced in the water column or at water-sediment interaction zone that is aerobic condition then it is diffused downward into the anaerobic sediment layer, where it is reduced to dinitrogen gas (Parick and Reddy, 1976).

Regarding to nitrogenous waste in pore water, release of ammonia appeared after day 10 of incubation due to decomposition of organic compounds and also diffusion from overlaying water (Binnerus *et al*., 1992; Burford and Longmore, 2001). Thereafter, ammonia in pore water was constant without a peak of nitrite while nitrate concentration had high fluctuation. In extracted sediment (Figure 4.4), ammonia remained at high concentration in the high salinity chamber (79 μg-N/g wet sediment) but it was low in the low salinity chamber (51 μg-N/g wet sediment). High ammonia content also indicated that sediment was rich of organic nitrogen compounds which could be converted to ammonia by mineralization or ammonification process.

4.4.1.2 Dissolved oxygen

Growth of autotrophic nitrifying bacteria and their activity are restricted in aerobic condition. Nitrification requires oxygen for the oxidation of ammonia and nitrite to nitrate as demonstrated in the below equation (Chen *et al*., 2006).

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7O_2N + 0.98NO_3^- + 1.041H_2O$$

$$+ 1.88H_2CO_3^-$$

$C_5H_7O_2N$ is the chemical expression of the bacterial cell of AOB. This equation indicated that nitrifying bacteria consumed oxygen for the cell biomass produced.

With this experiment, the oxygen concentration throughout the experimental period was almost stable. It was approximately 2.5 mg/L in control chambers and 7 mg/L in treatment chambers (Table 4.5). Thus, oxygen concentration was enough to support aerobic nitrification process which occurred at the overlaying water of the sediment. However, ORP value in sediment indicated that anaerobic denitrification process could be an co-occurrence process below the sediment layer. During the experiment, concentration of ammonia in the low DO chamber (control) was gradually increased to the highest concentration at day 5 while in the high DO concentration was at day 1 (Figure 4.10). Release of ammonia was due to the decomposition of organic matter. With this process, the oxygen is used by heterotrophic bacteria to decompose the organic matter in sediment. So, it was lead to the competition of the oxygen demand and growth between heterotrophic and nitrifying bacteria (van Benthum *et al*., 1997). With our result, ammonia in sediment chamber was rapidly eliminated when supplied with the high DO concentration at 7 mg/L. It was suggested that ammonification was greatly stimulated by the high DO concentration. This was disagree to the result of Rysgaard *et al.* (1994) which reported that ammonification in fresh water sediment from lake Vilhelmsbors S∅ was constant at different DO concentration. With the results in Figure 4.10A-B, sediment chamber with high DO could completely eliminated ammonia in 6 days which was 2 days faster than in chamber with low DO. Hence, it was indicated that ammonia oxidation was stimulated with the high DO. In addition, a constant of nitrate after day 10 of incubation was detected due to the efficiency of nitrated production by nitrification in overlaying water was probably equal to the efficiency of nitrate reduction by denitrification. Rysgaard *et al.* (1994) suggested that nitrate always diffused from the overlaying water into the sediment layer or it produced by the nitrifying bacteria in oxic sediment layer.

4.4.1.3 Inorganic carbon

Inorganic carbon is essential for nitrification process because nitrifying bacteria require inorganic carbon for autotrophic growth. In aquaculture pond, bicarbonate such as NaHCO3 was applied in order to increase the total alkalinity, maintain pH in the water and support growth of phytoplankton. However, maintaining proper alkalinity also provides bicarbonate for nitrifying bacteria that is the nitrogen biofilter in the pond. For the conversion of ammonia via nitrite to nitrate, nitrifying consumes alkalinity as revealed in the equation in section 4.4.2 (Chen *et al.*, 2006). It is indicated that nitrifying bacteria can used alkalinity for cell synthesis.

After adding $NaHCO_3$ to the treatment chambers at the C:N ratio of 2:1 (24.36 mg-C/L), the total alkalinity in the water was 130 mg/L. Figure 4.15 show trend of nitrogen conversion along the experimental period. Like the data of our previous study that was found the release of ammonia from sediment to water column. It was due to the decomposition of organic matter that leads to nitrogen mineralization (ammonification).

4.4.1.4 Organic carbon

There were several external carbon sources can be used as the electron donor such as starches, sugars and alcohols (Kessreu *et al*., 2003; Hamlin *et al*., 2008). However, methanol is a soluble organic carbon that is the most commonly used for enhancing denitrification systems. Based on the stoichiometric relationship of heterotrophic denitrification, the methanol to nitrate ratio have been reported in term of C:N ratio. The C:N ratio for complete denitrification process with methanol as a carbon source was between 3 to 10 (Ebeling *et al.*, 2006).

Figure 4.19A-C shows the change in inorganic nitrogen compounds in the water column. It was found that nitrogen cycle in the experimental chambers was forced by the natural processes including ammonification, nitrification and denitrification. Ammonia in control chambers (without methanol) was gradually released to the highest concentration and following by subsequently eliminated at day 18. With treatment-1 and treatment-2, after adding methanol to the C:N ratio of 2:1 and 4:1, respectively, ammonia was continuously released to the highest concentration that higher than control chamber and it was completely removed from the water at day

27 incubation (Figure 4.19A). Release of ammonia was due to the decomposition process (ammonification) of high organic matter sediment (Avnimelech and Ritvo, 2003; Moriaty, 1997). Increases of nitrite in all experimental units were found after ammonia oxidation (Figure 4.19B). However, nitrite was finally removed by the nitrite oxidation process that converts nitrite to nitrate (Figure 4.19C).

In general, nitrification can be found in both water column and at sediment surface but nitrification rate of water column and sediment is somewhat difficult to be compared due to the different unit of measurement. For example, nitrification rate in water column of Narragansett Bay and Chesapeake Bay was 0.16 and 0.45 mg-N/L/day, respectively (Berounsky and Nixon, 1993; Horrigan *et al*., 1990), while the nitrification rate in sediment of freshwater fish pond and polyculture fish pond was approximately 0.4-1 mg-N/m^2/day (Acosta-Nassar *et al*., 1994; Riise and Roos, 1997). As the activity of nitrifying bacteria were mostly found in biofilm, nitrification process was hence targeted mostly at sediment-water interactive zone with a minor portion in the suspended particles. As shown in Figure 4.19C, nitrate concentration in the chamber was almost constant throughout the experiment. Here, it was probably due to the coupling nitrification-denitrification processes in which nitrate from nitrification was further reduced by denitrification process. Other possibility was the anaerobic ammonia oxidation (anammox) process that ammonia and nitrite are converted to nitrogen gas in anoxic sediment (Rich *et al.*, 2008). However, anammox activity in natural condition with much lower ammonia and nitrite concentration than that found in wastewater treatment plant is rarely reported.

With this study, denitrification was occurred in both control and treatment chambers throughout the study period. This was indicated by ORP values between -265 to -364 mV in the sediment which was the potential of nitrate reduction (Li and Irvin, 2007). At an anoxic sediment layer, nitrate was reduced to nitrogen gas through a series of intermediate gaseous nitrogen oxide products (Ebeling, *et al*., 2006). Several studies suggested that addition of organic carbon could simulate denitrifying bacteria and other heterotrophic bacterial activities due to the fact that heterotrophic bacteria grew faster than autotrophic bacteria (Moriaty, 1997; Avnimelech and Ritvo, 2003; Cho and Molof, 20004: Ebeling, *et al*., 2006). Methanol is one of the most economical carbon source which is effective in supporting denitrification process. As nitrite concentration in the water was higher in

treatments than in control chambers, it could be assumed that addition of methanol into the sediment chamber promoted the ammonification process rather than the denitrification process.

Figure 4.20A-C shows that, ammonia in pore water of all experimental units was high at the initial day then it was decreased to lower than 15 mg-N/g wet sediment. In contrast, ammonia in extracted sediment from control chamber was higher than both treatment chambers (Figure 4.20D). Visscher and Duerr (1999) reported that decomposition of organic matter usually encounters in the flocculent sediment layer, approximately 2 cm above the sediment surface. The results indicated that decomposition of organic matter in the sediment rapidly released ammonia to the water column. Over 48 days incubation, concentration of nitrite in both pore water and extracted sediment of all experimental units were constantly low concentration (Figure 4.20B). Decrease of nitrate in sediment was found in all sediment chambers after incubated for 48 days (Figure 4.20B). Moreover, analysis of organic matter in the sediment showed that organic content in sediment was almost constant in all experimental units (Figure 4.21). In addition, trend of total oxidized nitrogen in sediment was decreased in control and in treatment-1 chambers while it was slightly increased in treatment-2 chambers (Figure 2.22). This was due to nitrogen dissimilation by bacteria (Greenwood, 1962).

4.4.1.5 Illumination

Conversion of inorganic nitrogen compounds in the dark sediment reactor was assumed as a sole bacterial activity while the illuminated sediment reactor revealed the activity of both bacteria and phytoplankton. To simulate organic nitrogen loading, 0.33 g of shrimp feed pellets (11 g/m^2) were added into both reactors in day 16. Increase of organic nitrogen loading was performed by adding 0.67 and 1.67 g shrimp feed pellets (22.33 and 55.66 g/m^2) at day 27 and 32, respectively. It was found that ammonia in the dark chamber was gradually released to water column due to ammonification process then following with nitrification-denitrification processes as it always found in the previous experiments. Nitrogen conversion in the illuminated chamber without extra loading of organic nitrogen was resemble the dark chambers. On the other hand, bloom of phytoplankton was found in illuminated sediment chamber at day 37 which was 5 days after high loading of

organic matter (1.67 g shrimp pellets feed or 10 mg-N/L). Higher ammonia and nitrite concentration were approximately two folds of that found in the dark reactor.

The results from this study illustrated that the major role of nitrogen conversion in aquaculture pond was through bacterial nitrification and denitrification processes rather than photosynthetic microorganisms. In general, growth of phytoplankton in aquaculture pond during the first week is presumably a result from ammonia release from sediment since ammonia-nitrogen is a major nutrient for phytoplankton growth (Raven *et al.*, 1992). Jangrassa *et al.* (2007) showed that increase of phytoplankton was negatively corresponded with the decrease of ammonia and phosphate in the water. With their study, artificial shrimp pond with low nitrification rate had higher chance of phytoplankton bloom than in the pond with high nitrification rate. The nitrogen assimilation process by only phytoplankton was not enough to carry waste from aquaculture activity. Massive bloom, on the other hand, is a common cause of water quality problem in aquaculture pond.

4.4.2 Effect of environmental factors on bacterial diversity in sediment chambers

In all experiments, changes of bacterial diversity were found in association with incubation period and environmental factors. Although the nitrifying bacteria was one of the most interesting bacteria in this study, isolation and identification of nitrifying bacteria was still under limitation due to it was a minor population of bacteria in the environment. Hence, nitrifying bacteria was never detected as an intense DNA band with PCR-DGGE using universal primer for total bacteria. Results of bacterial diversity in this study were illustrated by DGGE band pattern and diversity was analyzed by several indices.

4.4.2.1 Effect of salinity

In general, natural nitrifying bacteria including AOB and NOB distributes along fresh water to saline water. With traditional plate count method, density of AOB and NOB in water column of both low and high salinity sediment chambers was lower than those in sediment, nevertheless their density increased with time of incubation (Figure 4.6A-B). However, density of nitrifying bacteria in the

high salinity chamber was lower than in the low salinity chamber. It was similar to previous studies by Coci *et al.* (2005) that high salinity concentration induces salinity stress to bacteria cells due to it inhibited enzymes and their activity. With this study, SEM photographs showed that morphology of AOB was mainly rod-shaped bacteria and NOB was rod-shape and filamentous bacteria (Figure 4.7A-F).

Based on cultivation technique available for microorganisms, it is well known that only 0.01%–10.0% microbes on earth can be cultured and identified. There are only a few microbes that can be investigated by the pure culture method (Ward *et al*., 1990). Isolation of autotrophic AOB and NOB is difficult because of slow growth hence the appearance of colonies of such bacteria on a solid medium is slow, and the colonies are small (Prosser, 1986). With the molecular method, PCR-DGGE has been used to identify and evaluated the community of bacteria in natural habitat. Our result found that, with PCR-DGGE, most of the AOB and NOB isolated from sediment samples and kept in pure culture were not AOB or NOB but they were heterotrophic bacteria. For example, AOB-1 and AOB-3 colony was *Bacillus* sp. and *Micrococcineae* sp., respectively (Table 4.3). These bacteria were heterotrophic nitrifying bacteria. So, these bacteria can perform their nitrification activity in decline growth phase in associated with cell lysis (Lin *et al*., 2006; Sakai *et al*., 1997). Moreover, it could be presumed that the agar which was used in the selective media can also be decomposed and used as carbon and nitrogen sources by heterotrophic bacteria. Growth of autotrophic bacteria might be suppressed by overgrowth of heterotrophic bacteria. Hence, it is recommend that gellan gum that is a straight-chain polysaccharide might be used in stead of agar since many researches reported that the specific medium that suitable to autotrophic nitrifying bacteria must used gellan gum (Tomiyama *et al*., 2001; Tamaki *et al*., 2005).

With this experiment, bacterial diversity was analyzed only with sediment sample from the high salinity chamber (20 PSU). Freitag and Prosser (2003) reported that microbial community structure in sediment might be influenced by salinity changes and incubation time. This was in agreement with our result that at least six dominant bacteria species were disappeared after 31 days of incubation. Examples of those were bacteria band BAC-1 through BAC-4 (Figure 4.8) which all of them were closely related to Gammaproteobacteria (Figure 4.9). For this result, it

was indicated that *Marinobacter*, *Pseudomonas* sp., *Pseudomonas fluorescens* and uncultured bacterium clone G3DCM-151 are very sensitive to salinity. Because the original salinity of shrimp pond sediment used in this study was approximately 5 PSU so it was increased to 20 PSU after filling seawater into the chamber. This can lead to the salinity stress on bacterial community. Several studied have shown that the Gammaproteobacteria including *Colwellia*, *Halomonas* and *Pseudomonas* was found in broad range of marine sediment (Bissett *et al*., 2006; Torsvik *et al.*, 1996). In contrast, Abed *et al*. (2007) reported that bacteria groups of the Arabian Gulf including Cytophaga, Flavobacteria and Bacteriodetes were increased from the low to the high salinity.

Moreover, identification of 16S rRNA gene after PCR-DGGE analysis using the database in RDP II database with the Seqmatch program had lower similarity than that found with the BLASTN in NCBI database (Table 4.4). This was due to RDP II, when compared with NCBI, contained less stored data range of DNA sequence submitted by users (Ciantar *et al.*, 2005).

4.4.2.2 Effect of methanol addition

The community structure of the bacteria in sediment from all experimental units was examined by PCR amplification of 16S rDNA extracted from sediment followed by duplicate DGGE analysis. DGGE profile of 16S rRNA gene showed that bacterial community in the sediment was distinctly changed during 48 days study period (Figure 4.23). However, banding patterns of bacteria in treatment-1 and treatment-2 was almost similar after 48 days of incubation (Figure 4.24). So, it was suggested that the C:N ratio between 2:1 to 4:1 had slightly effect to bacterial diversity. Heylen *et al*. (2006) reported that the use of ethanol or succinate as a carbon source at the C:N ratio of 20 to 25 were suitable for the growth of denitrifying. With this study, changes of bacterial diversity were strongly affected by incubation period (Figure 4.23).

To explain or illustrates the change of bacterial diversity during the experiment, the Shannon-Weaver diversity index (*H*) was used in this study as it has been commonly applied in microbial ecology research (Hill, 2003; Eichner *et al*., 1999). A slightly decrease of diversity index was observed in all sediment chambers

after 48 day incubation. The diversity index was lowest in high organic carbon addition (treatment-2). These findings suggested that bottom soil contained fewer dominant bacterial species when incubated with the high C:N ratio and methanol addition decreased microbial diversity by stimulating specific group of heterotrophic bacteria (Moriaty, 1997).

Cluster analysis of the DGGE patterns with UPGMA method is presented in Figure 4.24. The UPGMA could be used to identify the similarity of DGGE banding patterns. With this study, UPGMA generated three major clusters of bacterial diversity which indicated that bacterial community in sediment chamber strongly differed after 48 days of incubation. On the other hand, bacterial community in day 48 of treatment-1 and treatment-2 was almost similar. Lucas and Hollibaugh (2001) reports that the addition of organic carbon sources as electron donors to the environment for selects the specific bacteria group; the result was found the differences between the initial and subsequent samplings of bacterial community.

The total of 16 interested DNA bands were excised from the gel (Figure 4.24) and the details are given in Table 4.9. DNA bands ORG-10, ORG-12 and ORG-15 appeared in all sediment chambers during the incubation period. With RDP II database, these were closely related to *Thermobispora bispora*, *Pseudomonas* sp. and Clone env. OPS17, respectively. While in NCBI database, these were higher similarity number which identified those bands as uncultured actinobacterium, uncultured bacterium and uncultured *Salegentibacter* sp., respectively. This was due to the RDP II database might not up to date and it is not contains a more diverse range of sequences (Ciantar *et al.*, 2005). In Figure 4.24, bands ORG-1, OGR-2, ORG-11, ORG-14 and ORG-15 appeared only at the initial day (day 0). After the total elimination of ammonia and nitrite in day 48, succession of new bacterial species was found in the sediment. These new DNA bands included ORG-3, ORG-4, ORG-6 to ORG-9. Moreover, intensity of DNA bands especially ORG-5 was increased in sediment chamber supplied with high methanol to the C:N ratio (4:1). Identification of ORG-5 revealed that is was belong to *Thioalcalovibrio denitrificans* of the γ-proteobacteria. This strain is denitrifying bacteria which would coexist with nitrifying bacteria due to the oxygen gradient in sediment chamber (aerobic condition in water column and anaerobic condition in sediment layer) and organic source from methanol addition. With this result, it was indicated that

methanol could promoted the growth and activity of denitrifying bacteria because decrease of nitrate concentration was also occurred in sediment chambers (Figure 4.19). In order to obtain more information for sediment bacterial groups, a phylogenetic distance tree for DNA bands ORG-1 to ORG-16 was analyzed, and shown in Figure 4.25. Six lineages were evaluated in sediment reactor including phylum Proteobacteria, Firmicutes, Actinomycetales, unclassified bacteria, Chloroflexi and Bacteriodetes. DNA bands ORG-3, ORG-5, ORG-7, ORG-8, ORG-2 and ORG-12 were grouped into Proteobacteria while all of them were most closely related to class γ-proteobacteria which comprised of facultative anaerobic and fermentative gram-negative bacteria (Aboutboul *et al*., 1995).

CHAPTER V

EFFECT OF SOIL DESICCATION ON NITROGEN COMPOUNDS CONVERSION AND MICROORGANISMS DIVERSITY IN SHRIMP POND SEDIMENT

5.1 Introduction

Earthen pond is the major type of aquaculture system. As mention in chapter 4, nitrogen conversion at sediment surface converts ammonia to nitrite and nitrate through nitrification process. This was followed by denitrification process in anaerobic sediment layer and the final product is nitrogen gas. In shrimp pond, sources of nitrogen input to are uneaten feed, fertilizers, organic waste excreted from animals, rainfall, water pumped into pond and nitrogen fixation by phytoplankton (Xia *et al*., 2004; Findlay *et al*., 1994). Only 20-30 % of nitrogen in feed can be converted into shrimp biomass while the rest is accumulated in water and in high organic content sediment at the pond bottom (Hargreaves, 1998; Burford and Williams, 2001). Accumulation of organic matters in sediment layer results in anaerobic conditions which produce several toxic substances such as ammonia, nitrite and hydrogen sulfide (Sonnenholzner and Boyd, 2000). Thus, the sediment–water interface in earthen ponds is a sink and a source of various substances that are potentially toxic for cultured species (Kassila, 2003).

Proper pond bottom management is one of the key successes of aquaculture in outdoor earthen pond. After each cultivating crop, water drainage then pond drying under direct sunlight is often performed by most farmers. This allows pond bottom soil to exposed with air and radiation from sunlight. With this procedure, water content is reduced, sediment pore is enlarged (Seo and Boyd, 2001; Avnimelech and Ritvo, 2003) and organic matter is rapidly oxidized (Apple, 1998). Nimrat *et al.* (2008) suggested that some of the pathogenic bacteria and microorganisms in the pond bottom soil could be eliminated after sun drying. Several researches reported that the nitrogen mineralization usually increase for a few days after the rewetting of dry soil due to soil microbial activities (Fierer and Schimel, 2002; Franzluebbers, *et al*., 1994; van Gestel *et al*., 1993). However, rapid change of soil moisture with the

rewetting may cause microbes to undergo osmotic shock (van Gestel *et al*., 1992). Moreover, UV radiation from sunlight is known as the harmful agent that affects aquatic ecosystems (Tuominen *et al*., 1999). Highly energetic UV has the potential for cell damage caused by direct effects on DNA and proteins and indirect effects via the production of reactive oxygen species in microorganisms in sediment. Communities of microorganisms are supposed to withstand a wide range of desiccation and sunlight exposure at different tolerance capability. This is considered as an important determinant of microbial communities in soil while microbial process in pond bottom soil after pond drying process is not yet fully investigated.

Propose of this study is to simulate aquaculture pond drying and determine the effect of soil desiccation on microbial process with emphasis on inorganic nitrogen conversion. The objective of this study was to determine ammonia removal rate, nitrite and nitrate conversion by nitrification process and changes of bacterial diversity after desiccation under both laboratory condition and outdoor artificial shrimp pond.

5.2 Materials and Methods

5.2.1 Sediment collection and preparation

Sediment sample from shrimp pond in Pathum Thani Province was transferred to the laboratory under cold and dark conditions (section 3.2). Thereafter, sediment samples were divided and placed on three trays. The first tray were persevered in refrigerator at 4°C, the second tray was left dry at room temperature under low light and the last tray was dried under direct sunlight exposure for one week.

5.2.2 Effects of pond bottom soil desiccation on inorganic nitrogen conversion and bacterial diversity under laboratory conditions

5.2.2.1 Experimental units

The experimental unit was a sediment chamber made of cylindrical PVC as described in section 3.3.1). There were three treatments of

sediment chambers with three replications. A first group was the control chamber packed with cold preserved sediment. A second group, which was assigned as treatment-1, was packed with the air-dried sediment. A last group (treatment-2) was packed with the sun-dried sediment. After that, each of those chambers was filled with 1.4 liters of 20 PSU seawater. Alkalinity of the water was adjusted to 130 mg-$CaCO_3$/L. The chambers were continuously aerated with sterilized air through a 0.22 µm filter and incubated at ambient air under dark condition.

5.2.2.2 Chemical and physical analysis

During 28 days of incubation, concentrations of ammonia, nitrite and nitrate in water column, pore water and extracted sediment, alkalinity, temperature, dissolved oxygen and pH of water and oxidation-reduction potential (ORP) at 2 cm below soil surface were regularly monitored. Organic content and total nitrogen in sediment were analyzed weekly. Detail of analytical methods is shown in section 3.4 and 3.5. The sediment samples from each experimental unit at days 0, 7, 14, 21, and 28 were collected and kept at -20ºC for bacterial diversity study.

5.2.2.3 Sediment bacterial analysis

Bacterial genomic DNA was extracted directly from sediment samples using FastDNA SPIN kit for soil (Qbiogene, USA). Fragments of 16S rDNA were amplified by using PRBA338f plus GC clamp and PRUN518r primers specific to all bacterial groups (universal primers set: Muyzer *et al*., 1993). Protocol of genomics DNA extraction and PCR-DGGE analysis is shown in section 3.7.1.3, 3.7.2.1 and 3.7.3.1. With this study, 10 selected DNA bands were cut, re-amplified and sequenced. The closest sequence similarity of the partial 16S rRNA gene sequences were determined by using the BLASTN (http://www.ncbi.nlm.nih.gov/) and Ribosomal Database Project (RDP) II (http://rdp.cme.msu.edu/). The phylogenetic tree was constructed using the Jukes-Cantor, Neighbor-joining program of PHYLIP in RDP II version 8.1 software.

With the DGGE banding patterns, diversity indices including species richness, evenness and the Shannon-Weaver index (Shannon and Weaver, 1949) were calculated by using the equations as mentioned in Chapter 3. In addition, the UPGMA was used for cluster analysis and dendrogram generation.

Ten nucleotide sequences reported in this study were deposited in the NCBI nucleotide sequence databases under accession numbers FJ595662 to FJ595671.

5.2.3 Effects of sun-drying on inorganic nitrogen conversion of sediment from shrimp pond under laboratory condition

This experiment was performed in order to evaluate the effect of sun-drying on nitrogen conversion capability of the sediment from shrimp pond. Sediment was collected from shrimp pond in Pathum Thani Province. The experimental units were separated into two groups. The first group was control chambers packed with untreated wet sediment. The second group was treatment chambers packed with sun-dried sediment. The drying was performed by exposing sediment sample to direct sunlight for 7 day until the sediment surface was completely dry.

After being packed with sediment, the chambers were filled with freshly prepared seawater with 20 PSU salinity. The alkalinity was adjusted to 130 mg-$CaCO_3$/L by sodium bicarbonate addition. Aeration with 0.22 µm filter-disc filtration was continuously supplied. The sediment chambers were incubated under laboratory condition and conducted with three replicates.

At the initial day of the experiment, all sediment chambers were supplied with ammonium chloride solution to the final concentration of approximately 2 mg-N/L. During 26 days experiment, water was regularly monitored for ammonia, nitrite and nitrate as described in Chapter 3. Alkalinity, pH and DO were occasionally monitored.

5.2.4 Effect of sediment drying on inorganic nitrogen conversion and microbial diversity in the outdoor artificial shrimp pond

5.2.4.1 Experimental setup and operation

Outdoor artificial shrimp ponds in the study were 500-liter plastic tanks (0.73 m^2 surface area) packed with sediments to the depth of 8 cm and filled with 450 L of 20 PSU seawater. Calcium carbonate was mixed into the tank in

order to adjust water alkalinity to 130 mg/L. These tanks were exposed to natural sunlight and covered with transparent plastic sheet for protecting rain. The experiment consisted of two control tanks containing wet sediment from shrimp pond and two treatment tanks containing sun-dried sediment. Sediment from shrimp pond in Pathum Thani was kept at 4˚C prior to use in control tanks. For treatment tanks, sediment was dried under direct sunlight for 7 days. Aeration was continuously supplied throughout the experimental period. To evaluate nitrogen conversion, ammonium chloride solution was added into all tanks to the final concentration at approximately 2 mg-N/L at day 12 of the experimental period.

5.2.4.2 Water quality analysis

During 26 days of the experiment, changes in ammonia, nitrite, nitrate, phosphate and Chlorophyll-*a* were daily monitored. Total nitrogen and organic-content in sediment was analyzed in days 0, 12, and 26. Dissolved oxygen, pH, salinity and alkalinity were occasionally detected in water while oxidation-reduction potential was measured both in water and in sediment. Phytoplankton was identified under light microscope. Detail of chemical analysis is shown in Chapter 3.

5.2.4.3 PCR-DGGE analysis of microorganism diversity

5.2.4.3.1 Bacterial community

Water and sediment samples were collected at day 0, 12 and 26 for bacterial diversity analysis using PCR-DGGE technique as mentioned in section 3.7.2.1. Primers PRBA338f-GC and PRUN518r that were specific to all bacteria groups were used. DGGE results were visualized after silver staining (section 3.7.3.1). Thereafter, selected DNA bands were collected, re-amplified and sequenced. The phylogenetic tree was constructed for bacteria sequences using the Jukes-Cantor and Neighbor-joining program of PHYLIP in the RDP II. The sequences were compared with available public data using the BLASTN tool at the NCBI and RDP II website. With DGGE profile from each sample, cluster analysis was determined by using the BIO-1D software (Vilber Lourmat, France). Several diversity indices including species richness, evenness and Shannon-Wiener index were also calculated.

Selected sequences were submitted to the GenBank database (GenBank accession number: FJ665393 to FJ665398).

5.2.4.3.2 Ammonia-oxidizing bacteria (AOB) community

Diversity of AOB in sediment was analyzed using the AOB-specific primers set, CTO 189f-GC (a mixture of three forward primers) and CTO654r primer (section 3.7.2.2). DGGE gels were visualized by silver staining. Selected DGGE bands were cut, re-amplified and sequenced. Bacterial species of the sequences were revealed by the BLASTN in NCBI website.

5.2.4.3.3 Eukaryotic plankton community

In this study, PCR-DGGE analysis was applied to determine the diversity of eukaryotic organisms in water at day 6, 12 and 26. Detail of DGGE analysis is shown in section 3.8. Genomic DNA in water samples was extracted with DNA Extraction kit for Plant, amplified with primers 1427f plus GC clamp and 1616r, and separated by DGGE. The gel was stained with a Silverstar Staining kit and selected DNA bands were cut, re-amplified and sequenced. With DGGE profile from each sample, phylogenetic tree, cluster analysis, species richness, evenness and Shannon-Wiener index were calculated.

Ten DNA sequences of eukaryotic microorganisms found in this study were submitted to GenBank under accession numbers FJ713792 to FJ713801.

5.2.5 Ammonia removal efficiency of outdoor artificial shrimp pond

In addition to previous experiments, ammonia removal efficiency was evaluated in simulated shrimp pond with consequential ammonia addition. With this study, nitrogen loading in the experimental system was simulated by pulse addition of 0.5 mg-N/L ammonium chloride into the water on daily basis. This was an attempt to simulate nitrogen loading of shrimp culture at approximately 40 shrimps/m^2 (20 g of shimp/m^2 fed with 38% protein feed at 5% body weight/day). This loading was equal to 225 mg-N/tank/day or 296.1 mg-N/m^2/day. After 7 days of the experiment which

ammonia was accumulated in all tanks, the experiment was prolonged for another 14 days until ammonia and nitrite concentrations were decreased below 0.1 mg-N/L. Then, low concentration of ammonia loading at 0.2 mg-N/L/day was applied to all tanks. This was the simulation of nitrogen released from shrimp culture at approximately 20 shrimps/m^2. This nitrogen loading was equal to 90 mg-N/tank/day or 118.4 mg-N/m^2/day. During the experiment, water and sediment quality was regularly monitored as described in section 3.5.1.1-4.

5.3 Results

5.3.1 Water and sediment qualities in shrimp pond

In this study, sediment was collected from shrimp pond in Pathum Thani province. The sediment was collected during shrimp cultivating crop at approximately 3 months. Chemical and physical parameters of shrimp pond were measured during sediment sampling. Concentrations of ammonia, nitrite and nitrate in water, pore water and extracted sediment are presented in Table 5.1. There was low ammonia, nitrite and nitrate in water column. In contrast, average nitrate concentration was high in pore water while average ammonia concentration was high in extracted sediment. For nitrite concentrations in both pore water and extracted sediment were substantially low. Salinity, pH and total alkalinity of water in the pond were 5 PSU, 7.67 and 140 mg-$CaCO_3$/L, respectively. DO in the water was 4.6±0.8 mg-O_2/L and ORP in sediment was –169.3±34.78 mV. Soil texture was 58% clay, 36% slit and 6% sand.

Table 5.1 Average concentrations of ammonia, nitrite and nitrate (Mean±SD, N=3) in the water column, the pore water and the extracted soil of a shrimp pond.

Nitrogen compounds	Water column (mg-N/L)	Sediment	
		Pore water (mg-N/g wet sediment)	Extracted sediment (mg-N/g wet sediment)
Ammonia	0.26±0.04	7.47±0.30	49.07±7.33
Nitrite	0.02±0.00	0.90±0.37	0.49±0.11
Nitrate	1.08±0.02	37.44±0.61	4.24±0.23

5.3.2 Effect of sediment drying on inorganic nitrogen compound conversion and bacterial diversity under laboratory conditions

In this study, sediment in control chambers was wet sediment from shrimp pond, treatment-1 was air-dried sediment, and treatment-2 was sun-dried sediment. The chambers were filled with 1.4 L of 20 PSU water and incubated under laboratory conditions for 28 days. Environmental parameters including DO, pH, temperature and alkalinity in water, and ORP in both water and sediment are shown in Table 5.1. Due to continuous aeration, DO was constant at between 7.9 and 8.2 mg-O_2/L throughout the experimental period. ORP in water column was between 99.2 and 276.6 mV while ORP in sediment was between -157.0 and -60.7 mV. Total alkalinity of water was almost constant in control chambers but alkalinity in treatment-1 and 2 was decreased after 28 days of experiment. Water content in sediment was increased with time (Figure 5.1) in all chambers. Sun-dried sediment had lowest water content in the first day but the water content rose rapidly to the same concentration as the air-dried sediment until the end of the experiment.

Table 5.2 DO, ORP, pH, and alkalinity (Mean±SD) of the sediment in the experimental chambers during the incubation period (28 days). The shown data were Mean±SD and data in the bracket is maximum and minimum values.

Parameter	**Control (wet)**	**Treatment-1 (air-dried)**	**Treatment-2 (sun-dried)**
DO (mg-O_2/L)	8.9±0.5	8.8±0.5	8.7±0.5
	(9.2, 8.0)	(9.2, 8.2)	(9.1, 7.9)
ORP in water (mV)	136.8±25.0	191.6±68.5	179.4±39.7
	(162.6, 99.2)	(275.6, 128.2)	(224.9, 129.8)
ORP in sediment (mV)	(-117.3)±30.3	(-95.8)±22.5	(-93.4)±19.7
	(-82.8, -157.0)	(-73.6, -122.5)	(-60.7, -113.5)
pH	8.87±0.1	8.33±0.1	8.50±0.1
	(8.8, 8.6)	(8.39, 8.32)	(8.6, 8.4)
Temperature (ºC)	25.8±1.1	25.9±1.1	25.8±1.1
	(27.3, 24.7)	(27.3, 24.7	(27.0, 24.5)
Alkalinity (mg/L)	100, 90	100, 30	100, 60
	(Day 0, 28)	(Day 0, 28)	(Day 0, 28)

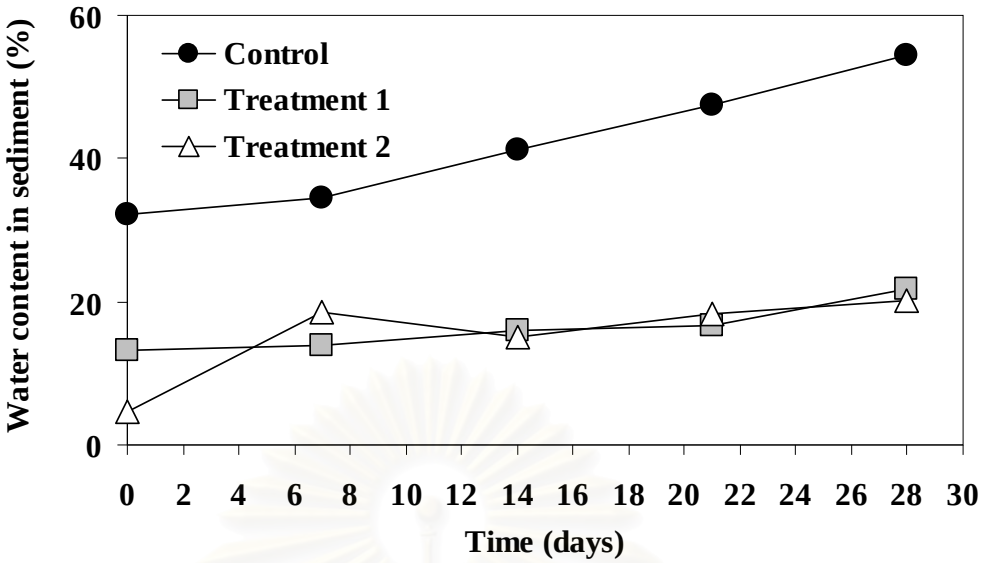


Figure 5.1 Change in water content in sediment during incubated for 28 days.

5.3.2.1 Water and sediment quality

Conversions of ammonia, nitrite and nitrate in water are shown in Figure 5.2A-C. Release of ammonia from sediment to water column was detected in control and treatment-2 chambers. During the first two days, ammonia in control chambers was gradually increased up to the highest concentration of 1.69±0.1 mg-N/L (Figure 5.2A). This was similar to treatment 2 chambers that ammonia was released after filling seawater and the concentration increased to the highest at 0.63±0.3 mg-N/L in day 3. However, peak of ammonia was not found in air-dried sediment (treatment-1). Nitrite in all experimental chambers was found after ammonia oxidation (Figure 5.2B). There was high nitrite accumulation both in control and treatment-2 chambers. The highest concentration of nitrite in control and in treatment-2 chambers was 2.31±0.24 mg-N/L at the day 18 and 2.06±0.3 mg-N/L at day 14, respectively, while nitrite in treatment-1 was only 0.77±0.39 mg-N/L at day 7 of the experiment then it was totally eliminated in day 11. The highest nitrate concentration was found in treatment-2 chambers which fluctuated between 2.38 and 3.64 during a day 8 to 28. On the other hand, nitrate in treatment-1 was steadily low between 0.74 - 1.57 mg-N /L throughout the experiment (Figure 5.2C).

Figure 5.3 shows that ammonia in pore water during the first half of experiment was rather constant. Increase of ammonia in pore water was found in

all chambers during the second half of the experiment whereas ammonia in water column was substantially low. This indicated the activity of ammonia oxidizing bacterial that rapidly convert ammonia in pore water to nitrite. Consequently, high nitrite concentration was found in pore water of control chambers during day 6-14. This related with an increase of nitrite in water column of the control chambers (Figure 5.3B). Sun-dried sediment had the highest ammonia concentration of 7.85 mg-N/g wet sediment at the initial day. Ammonia in extracted sediment was decrease to below 15 mg-N/g wet sediment after one week and the concentration remained constant until the final day. Nitrite and nitrate in extracted sediment from all experimental units were constantly low throughout the experiment. Moreover, as shown in Figure 5.4-5, total nitrogen and organic content in sediment from all chambers was somewhat stable throughout 28 days experiment.

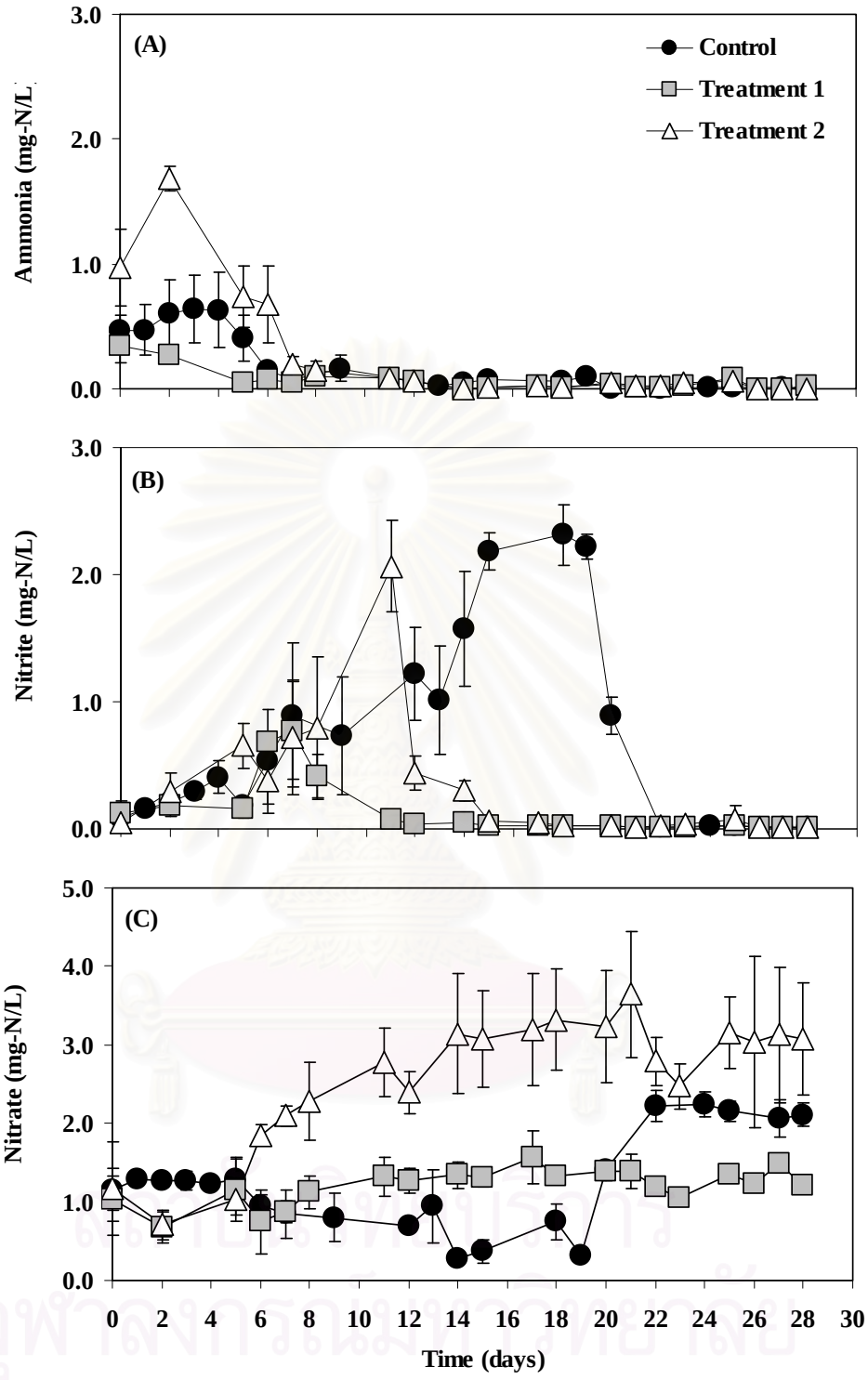


Figure 5.2 Changes in ammonia (A), nitrite (B) and nitrate (C) in water column of the sediment chambers during 28 day incubation. Control chamber was packed with fresh sediment from shrimp pond. Treatment-1 and 2 chambers were packed with air-dried and sunlight-dried sediment, respectively.

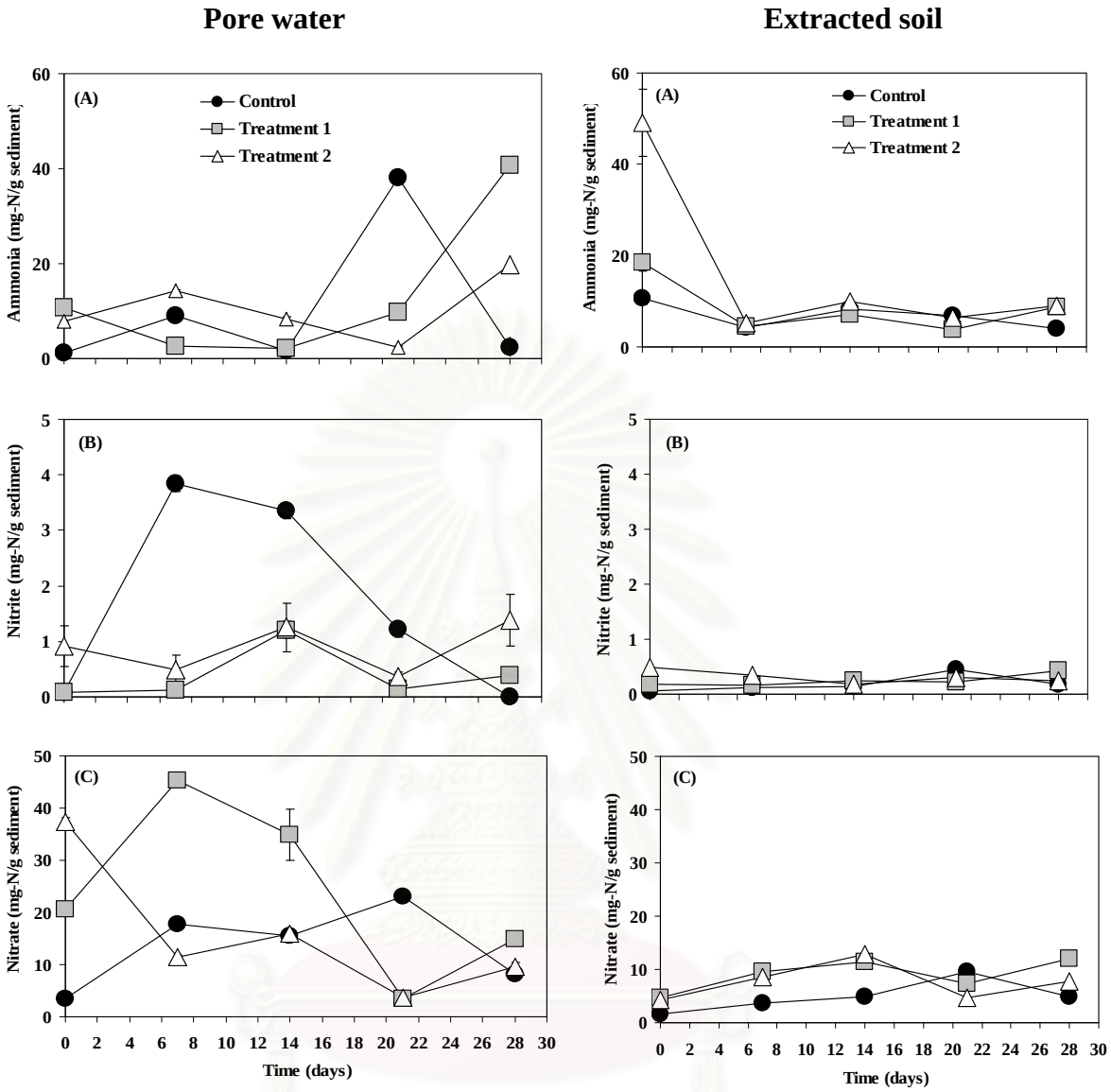


Figure 5.3 Concentrations of ammonia (A), nitrite (B) and nitrate (C) of pore water and of extracted soil during 28 day incubation. Control chambers were packed with fresh sediment from shrimp pond. Treatment-1 and 2 chambers were packed with air-dried and sunlight-dried sediment, respectively.

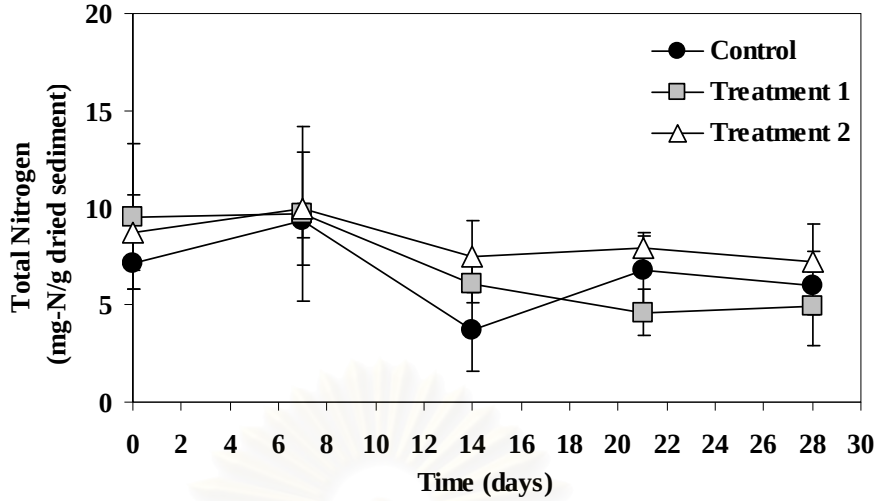


Figure 5.4 Changes in total nitrogen of sediment chambers during 28 day incubation.

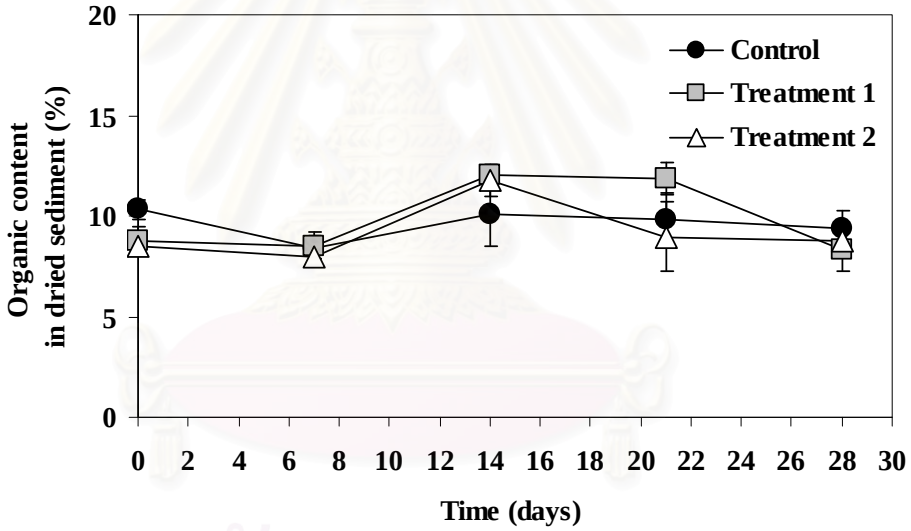


Figure 5.5 Changes in sediment organic content of sediment chambers during 28 day incubation.

5.3.2.2 Sediment bacterial diversity

At day 0, 7, 14, 21 and 28, sediment samples were collected from control, treatment-1 and treatment-2 chambers for PCR-DGGE analysis. Figure 5.6 reveals the DGGE banding patterns of 16S rRNA gene fragments obtained after PCR amplification of sediment samples. With the DGGE banding patterns of samples at the initial day, the number of dominant DNA bands obtained from control, treatment-1 and 2 chambers was 20, 18 and 14 bands, respectively. As the result, bacterial diversity in wet sediment from control chambers had more diverse than those found in dried sediment of both treatments-1 and 2. The lowest bacterial diversity was revealed in the sun-dried sediment (treatment-2). DGGE banding pattern during the initial day in Figure 5.6 indicated that band 1, 3 and 4 were presented in both control and treatments while band 5 and 7 was not found in dried sediment (treatment-1 and 2). Changes of bacterial diversity in the sediment were induced by experimental conditions and incubation time. Diversity of bacteria in control and treatment-1 was slightly reduced after 28 day incubation whereas that of treatment-2 was rather stable.

Cluster analysis of the DGGE patterns using UPGMA method is presented in Figure 5.7. The result shows that bacterial community in sediment chambers with different incubation time could be separated into two groups. At the initial day (day 0), bacterial communities of control and treatment-1 were rather similar with 86% similarity, but they were obviously different from that in treatment-2 (57% similarity). After 28 day incubation, diversity of bacteria in control chambers could be separated from cluster of treatment-1 and 2 with the similarity of 70 and 77%, respectively.

Total numbers of DNA bands and their relative intensities in each lane were detected in order to calculate the bacterial diversity index and the results are shown in Figure 5.8A-C. It was found that the highest Shannon-Weaver diversity index was in control chamber at the initial day while the lowest was in treatment-2 at day 7. After 28 day incubation, the diversity index was slightly decreased and not apparently different among all experimental units. The species richness in both control and treatments was fluctuated with the experimental period.

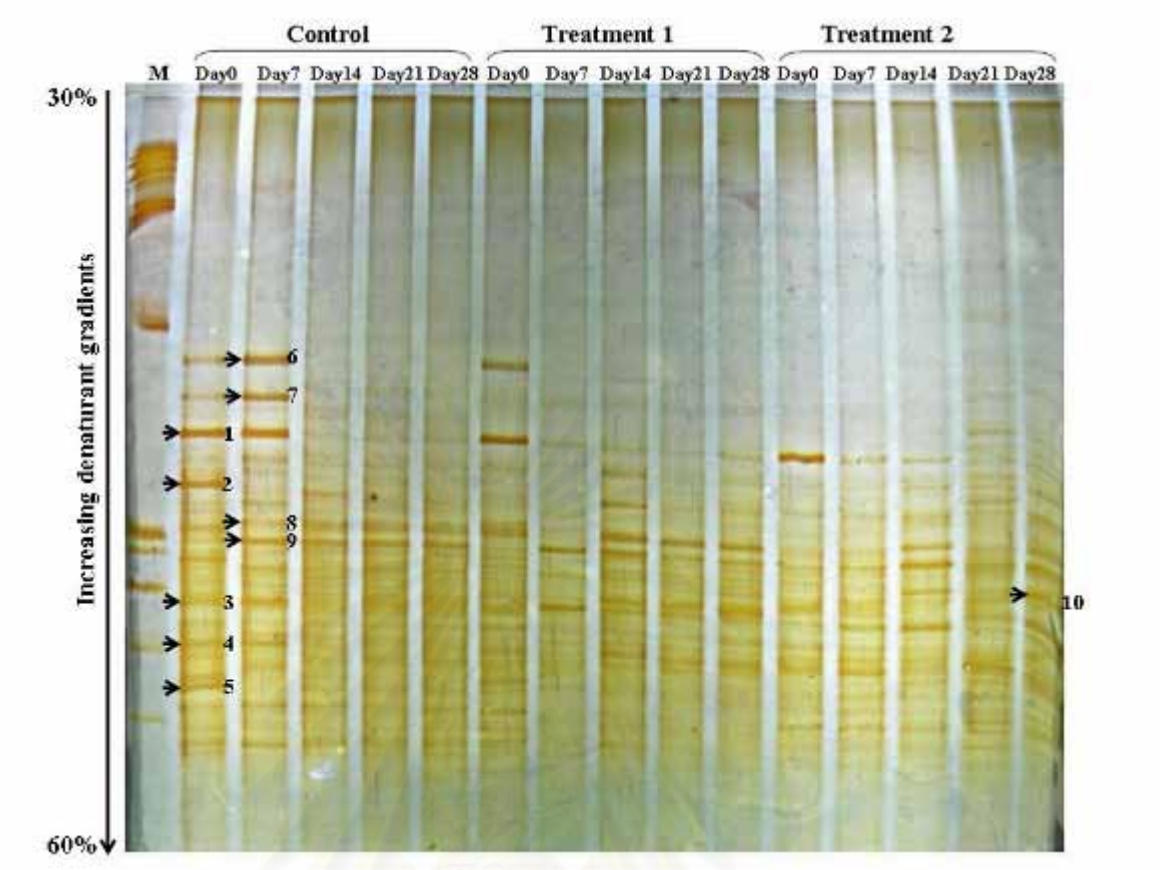


Figure 5.6 DGGE bacterial profile in sediment chambers incubated for 28 days. 16S rRNA gene was amplified with primers 338f plus GC clamp and 518r and strained with silver staining method. Lane M: DNA ladder (ϕX174 DNA-HaeIII digest, Biolab, UK). Control chambers were packed with fresh sediment from shrimp pond while treatment-1 and 2 chambers were packed with air-dried and sunlight-dried sediment, respectively. Arrows represents the selected DNA bands as follows: band 1 to 10 were re-amplified and sequenced. DNA bands: 1, DES-1; 2, DES-2; 3,DES-3; 4,DES-4; 5,DES-5; 6,DES-6; 7,DES-7; 8,DES-8; 9,DES-9; 10,DES-10.

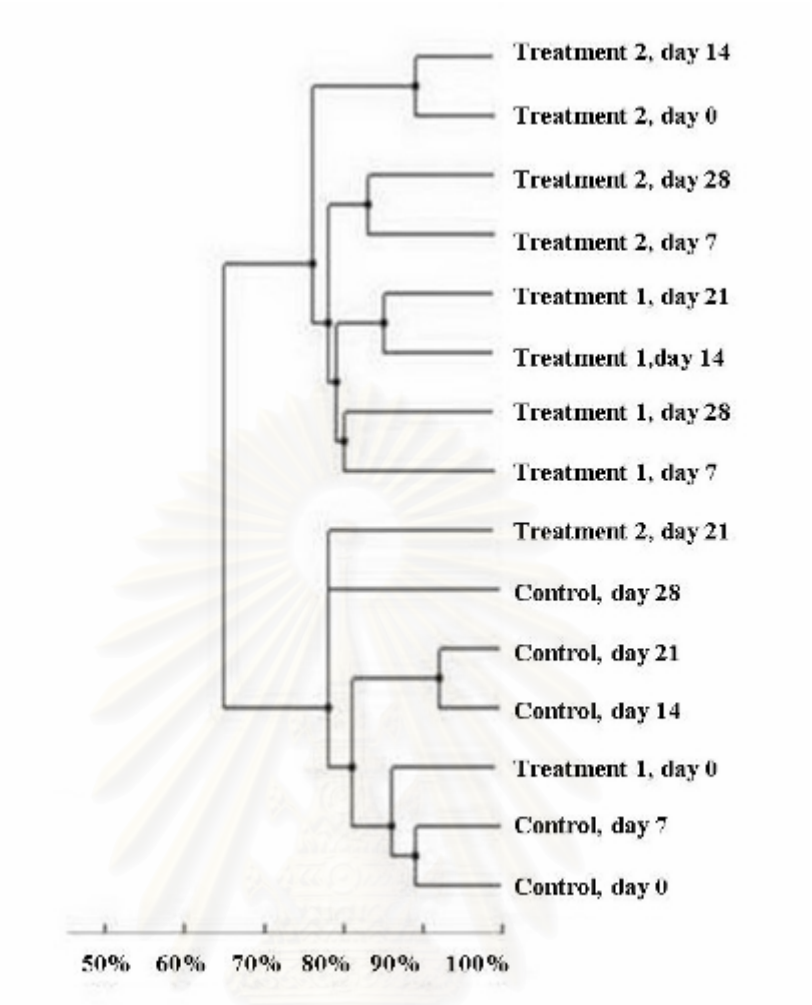


Figure 5.7 The UPGMA dendrogram shows the clustering analyses of digitized DGGE profile (Figure 5.6). Cluster analysis is based the similarity of the DGGE banding patterns as described in Chapter 3. Scale bar indicates Nei and Li's similarity coefficient (95% of confidence).

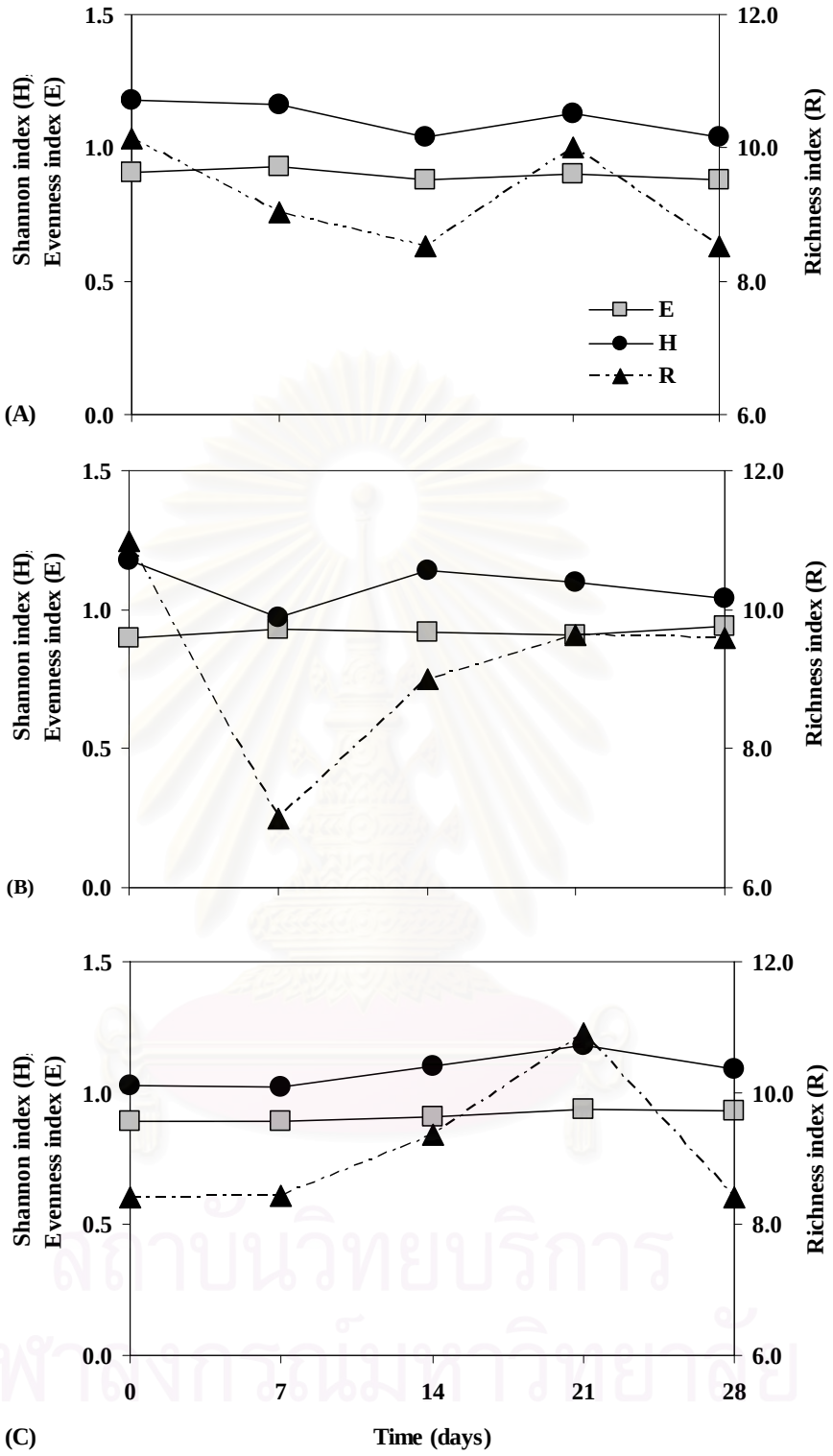


Figure 5.8 Species richness, evenness, and Shannon-Weaver index of bacteria from control chamber (A), treatment-1 chamber (B), and treatment-2 chamber (C) operated under laboratory conditions at day 0, 7, 14, 21 and 28 of experimental period. All values were calculated from the DGGE banding in Figure 5.6.

The identity of the selected DNA bands (band 1 to 10) was confirmed by direct sequencing the re-amplified 16S rRNA gene fragments (Figure 5.6). The closest sequence similarity of selected DNA bands is listed in Table 5.3. DNA bands 1, 3 and 4 that appeared in all sediment samples at the initial day was identified as Clone VC2.1, Bac 4 and Clone SB-17 of *Bacillus horti*, respectively. According to the database in NCBI, all of the obtained sequences were mostly related to uncultured bacteria. Some of DNA bands that disappeared in dried sediment were bands 5 and 7. DNA band 5 was mostly related with *Nitrosomonas* which is a group of ammonia oxidizing bacteria with the identity of 99% while DNA band 7 had 97% identity to *Methylophaga*. Bacterium in the sediment that was disappeared after sun-dried was DNA band 6 which was identified as *Methylomonas* (96 % identity). DNA band 10 that was found in all experimental conditions was closely related with *Vibrio* (94% identity).

Total of 10 different DNA sequences were analyzed with a Jukes-Cantor model then the phylogenetic tree was constructed by neighbor-joining method. Figure 5.9 shows the distance tree for DNA band 1 to 10 labeled as DES-1 to DES-10. These DNA bands from sediment chambers were evaluated as three lineages including phylum unclassified Firmicutes, Proteobacteria and unclassified Bacteriodetes. The phylogenetic tree revealed that the majority of the obtained sequences were mostly related to Proteobacteria with three subclass including γ-proteobacteria, β-proteobacteria and Episoproteobacteria.

Table 5.3 Sequence similarities of the interested DNA bands appearing in the gel of DGGE (Figure 5.6).

DNA band	RDP II data base			NCBI data base		
	ID	Description	Identities (%)	ID	Description	Identities (%)
1	B.horti1	*Bacillus horti* str.K13 JMC 9943	60	EF613803	Uncultured bacterium clone GA79	100
2	AB030931	*Bacillus* Ipso2 str. IPS2	59	EF613803	Uncultured bacterium clone GA79	98
3	AF068786	Clone VC2.1 Bac 4.	82	DQ909764	Uncultured bacterium clone FS396_454_1000bp_1147B	99
4	AF029044	Clone SB-17	83	EU440064	Uncultured marine bacterium isolate DGGE band 2(4)	98
5	Nmn. marina	*Nitrosomonas marina* str. C-56; Nm63.	73	EF092216	Uncultured *Nitrosomonas* sp. clone B2505_H7	99
6	Mlm.sp LW15	*Methylomonas* str. LW15	71	AF150794	*Methylomonas* sp. LW15	96
7	Mp.marina	*Methylophaga marina*	71	AM238560	Uncultured *Methylophaga* sp. partial clone SARG_19	97
8	AB015253	str. JTB254	88	AB015253	Uncultured gamma proteobacterium clone JTB254.	98
9	AJ011042	str. R1	71	AM409969	Uncultured bacterium clone d6-3.	95
10	V.mytili_1	*Vibrio mytili*	67	DQ978991	*Vibrio* sp. S-10	94

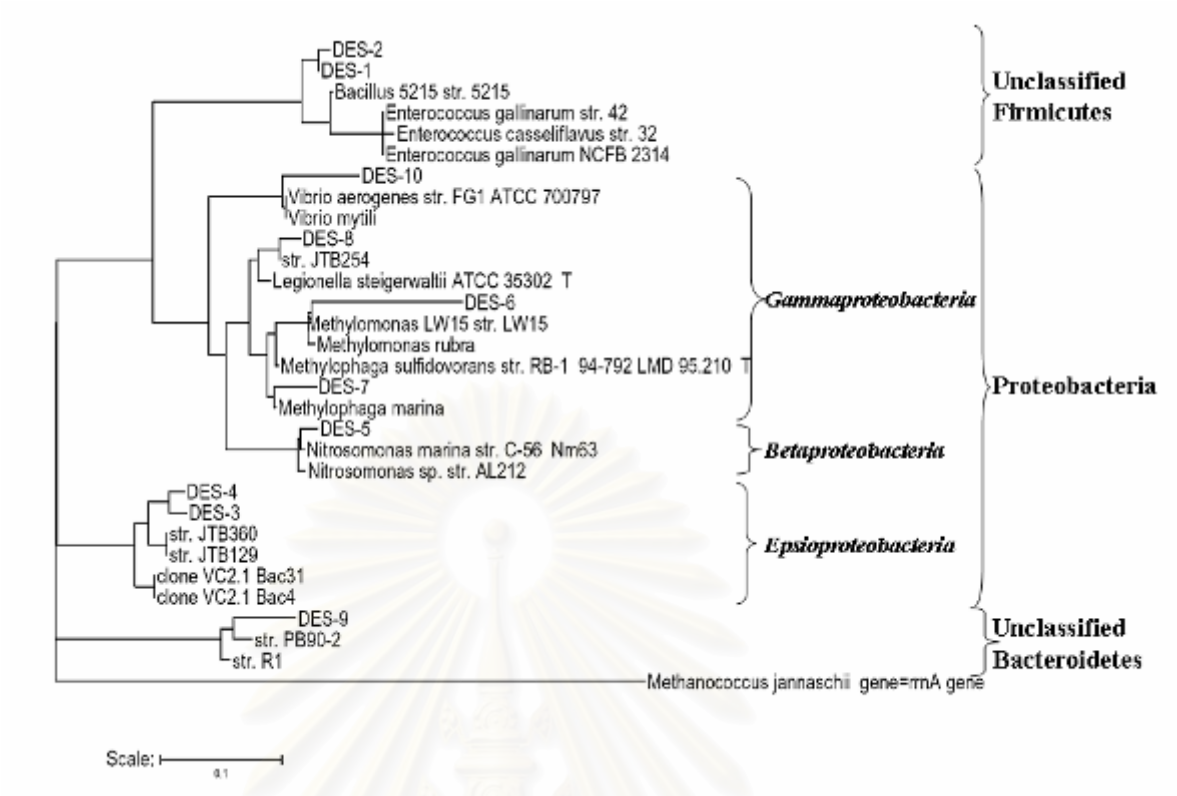


Figure 5.9 Phylogenetic trees demonstrating the relationship of ten interested DNA band sequences (DES-1 to DES-10) from the gel (Figure 5.6). The tree was constructed by using maximum likelihood criteria and the neighbour-joining method (RDP II version 8.1 software online packages with its database). *Methanococcus jannaschi* was used as outgroup. The bar represents 0.1 nucleotide substitution per position.

5.3.3 Effect of sun-dry on ammonia removal efficiency of shrimp pond sediment under laboratory conditions

In this study, ammonia removal efficiency of wet and dry sediment samples was evaluated. The experimental units were 1.4 L cylinder shape chambers with 11 cm diameter packed with wet or sun-dried sediment samples collected from shrimp pond. The experiment was started by adding ammonia-nitrogen to the initial concentration of 2 mg-N/L. DO, ORP, pH, temperature and alkalinity were occasionally determined during 26 day incubation. It was found that average DO and temperature in the water during 26 days of the experimental period were 6.9 mg/L and

27.3˚C respectively. ORP in sediment was higher in treatment chambers than that in control chambers while pH in treatment chambers was lower than control chambers. Total alkalinity was slightly decrease in treatment chambers while it was unchanged in control chambers (Table 5.4).

Table 5.4 Environmental parameters during 26 day incubation. The shown data were Mean±SD and data in the bracket is maximum and minimum values. Control chambers were packed with wet sediment while treatments were packed with sun-dried sediment.

Parameter	**Control-1**	**Control-2 (+ NH_4Cl)**	**Treatment-1**	**Treatment-2 (+ NH_4Cl)**
DO ($mg\text{-}O_2/L$)	6.9±-0.5 (7.7, 6.4)	7.0±0.4 (7.66, 6.57)	6.9±0.5 (7.6, 6.3)	6.9±0.5 (7.6, 6.4)
ORP in water (mV)	234.7±46.3 (274.0,147.8)	229.1±53.0 (273.9, 128.8)	256.3±32.9 (286.9, 198.3)	248.5±39.5 (283.2, 174.8)
ORP in sediment (mV)	(-101.3) ±52.7 (-81.7, -246.8)	(-129.9) ±55.7 (-91.9, -291.9)	(-45.3) ±30.4 (-3.40, -68.90)	(-45.2)±39.2 (-2.20, -78.50)
pH	8.3±0.2 (8.5, 8.0)	8.3±0.1 (8.5, 8.0)	8.1±0.2 (8.4, 8.0)	8.1±0.2 (8.3, 7.9)
Temperature (ºC)	27.3±0.4 (28.1, 8.0)	27.2±0.4 (28.0, 27.9)	27.4±0.5 (28.2, 26.0)	27.4±0.5 (28.2, 26. 7)
Alkalinity (mg/L)	130, 120 (day 0, 26)	130, 130 (day 0, 26)	130, 110 (day 0, 26)	130, 100 (day 0, 26)

After filling seawater into the control sediment chamber, ammonia concentration at initial was 0.38±0.10 mg-N/L. Thereafter, ammonia concentration was decreased to below 0.1 mg-N/L in 7 days (Figure 5.10A). Concentration of nitrite and nitrate in control chambers during the experiment were less than 0.2 and 1.9 mg-N/L, respectively (Figure 5.10B-C). In contrast, peaks of ammonia and nitrite occurred in sun-dried sediment chamber (treatment-1). Ammonia concentration

increased to the highest peak of 3.34±0.30 mg-N/L in day 8 then decreased to below 0.08±0.05 mg-N/L at day 12. Nitrite peak was found after day 9 with the highest concentration of 5.28±0.70 mg-N/L in day 14. Nitrite was finally decreased to below 0.05 mg-N/L in day 22. Moreover, during day 14 to 24, nitrate concentration was increased from 0.02 to 5.58 mg-N/L. It has to be noted that nitrate accumulation in the sediment chamber was an unusual phenomenon because it had never been found in any of the previous experiments.

With ammonia addition, initial concentrations of ammonia in control-2 (wet sediment) and in treatment-2 (sun-dried sediment) chambers were 2.17±0.38 and 2.41±0.19 mg-N/L, respectively. Thereafter, ammonia in control-2 was continuously decreased to 0.13 mg-N/L in day 6 while nitrite and nitrate were remain constant at low concentration throughout 26 days experiment (Figure 5.9A-C). Unlike the control-2, an increase of ammonia to the highest concentration of 5.04±0.42 mg-N/L in day 5 was found in treatment-2. Then, ammonia peak was replaced by a nitrite peak during day 8-22 with the highest nitrite concentration of 6.80±0.63 mg-N/L. Because sediment in treatmen-2 was the sun-dried sediment, unusual accumulation of nitrate was also found. The highest nitrate concentration of treatment-2 was 6.25±0.56 mg-N/L in day 24.

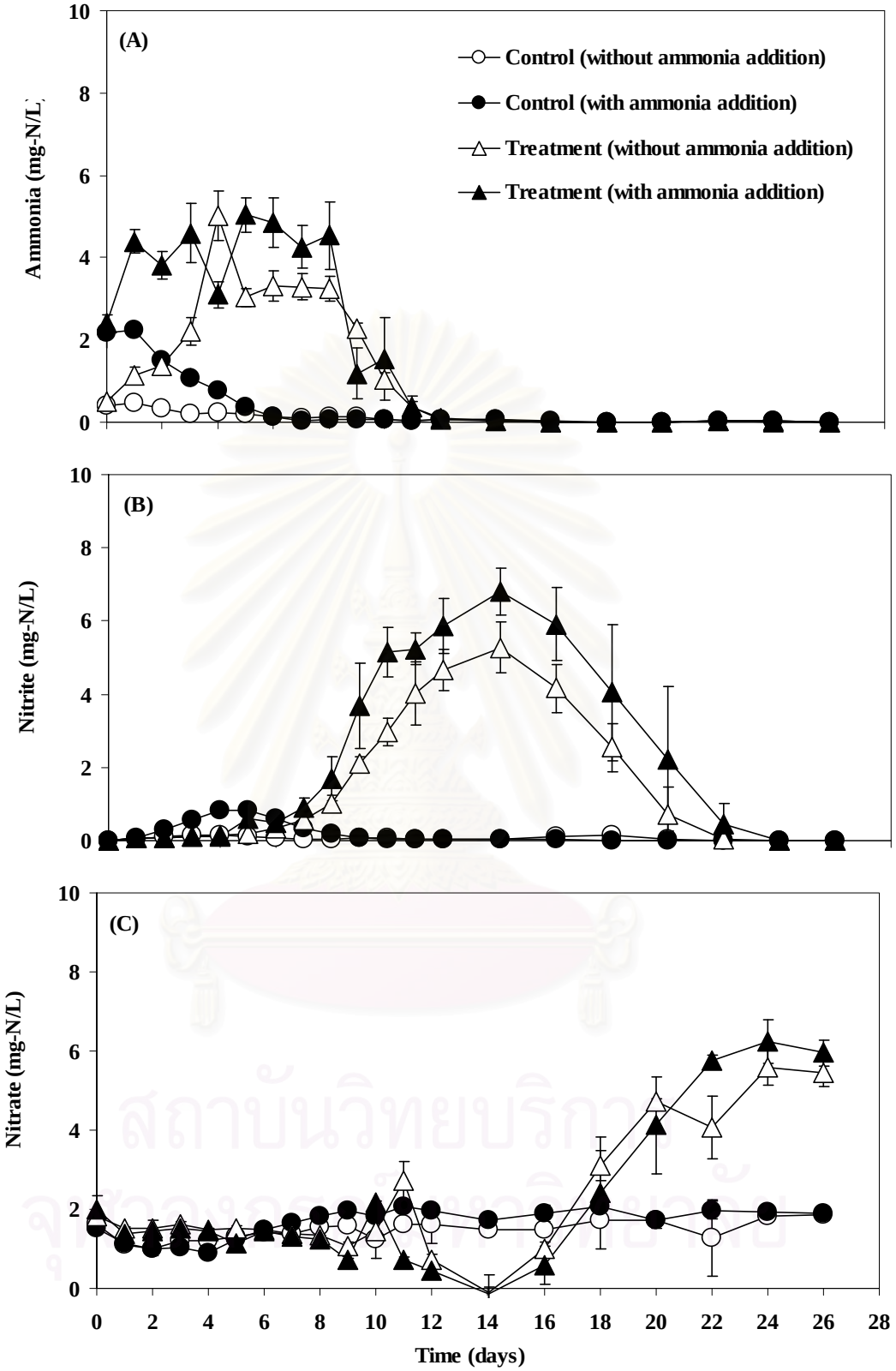


Figure 5.10 Changes of ammonia (A), nitrite (B) and nitrate (C) in sediment chambers incubated under dark condition for 26 days. Controls were wet sediment and treatments were sun-dried sediment.

5.3.4 Effect of sediment drying on inorganic nitrogen conversion and microbial diversity in the outdoor artificial shrimp pond

The experiment consisted of control tanks packed with untreated wet sediment from shrimp pond and treatment tanks with sun-dried sediment. Table 5.5 shows environmental parameters such as DO, ORP, light intensity, salinity, pH and alkalinity during the experiment. It was found that dissolved oxygen in all tanks was between 6.6-7.6 mg-O_2/L due to continuous aeration. Oxidation-reduction potential (ORP) in water fluctuated between 117.1 and 289.2 mV while ORP in sediment was between -30.0 and -156.0 mV. All artificial shrimp pond was exposed to natural sunlight but shading was provided by transparent plastic sheet for rain protection. Water temperature during the experiment was between 25.0 and 27.1ºC. Average salinity of treatment tanks was 23 PSU which was slightly higher than 20.6 PSU in control tank. Decrease in alkalinity during the experiment was found in both control and treatment tanks (Table 5.5).

5.3.4.1 Water and sediment quality of artificial shrimp pond

Figure 5.11A-C shows the concentration of ammonia, nitrite and nitrate of the 26 days experiment. During the first half (day 0-12) of the experiment, treatment tanks containing sun-dried sediment released higher ammonia concentration (0.35 mg-N/L) into the water column while ammonia in control tank was only 0.07 mg-N/L. After ammonium chloride addition in day 12, ammonia in treatment tanks was removed at higher rate than control. For nitrite, increase of nitrite in water column of treatment tanks (0.2 mg-N/L) was found after the decline of ammonia. Small peak of nitrite was also found following ammonium addition in day 12 but the maximum nitrite concentration (0.3 mg-N/L) was quite low in comparison with an addition of 2.4 mg-N/L ammonium chloride. Nitrate concentration in control tanks was quite constant during the first 12 days but it was slightly increase after ammonia addition in day 12.

Table 5.5 Environmental parameters in artificial shrimp ponds during 26 days of experiment. The data show Mean±SD with maximum and minimum value in the bracket.

Parameter	**Control tank**	**Treatment tank**
DO in morning (mg-O_2/L)	7.2±0.2	7.1±0.3
	(7.6, 7.0)	(7.6, 6.6)
ORP in water (mV)	195.7±56.3	200.7±61.4
	(267.2, 118.6)	(289.2, 117.1)
ORP in sediment (mV)	(-105.4)±33.7	(-71.0)±26.1
	(-70.5, -156.0)	(-33.0, -107.1)
Light intensity in morning (Lux)	4913±2203	5314±2090
	(8100, 1800)	(9090, 3100)
Temperature in water (ºC)	26.1±0.8	26.3±0.7
	(27.1, 25.0)	(27.1, 25.1)
Salinity (PSU)	20.6±0.5	23.0±0.9
	(20, 21)	(21, 24)
pH	8.1±0.2	8.0±0.2
	(8.4, 7.9)	(8.3, 7.7)
Alkalinity (mg/L)	120, 65	120, 60
	(Day 0, 26)	(Day 0, 26)

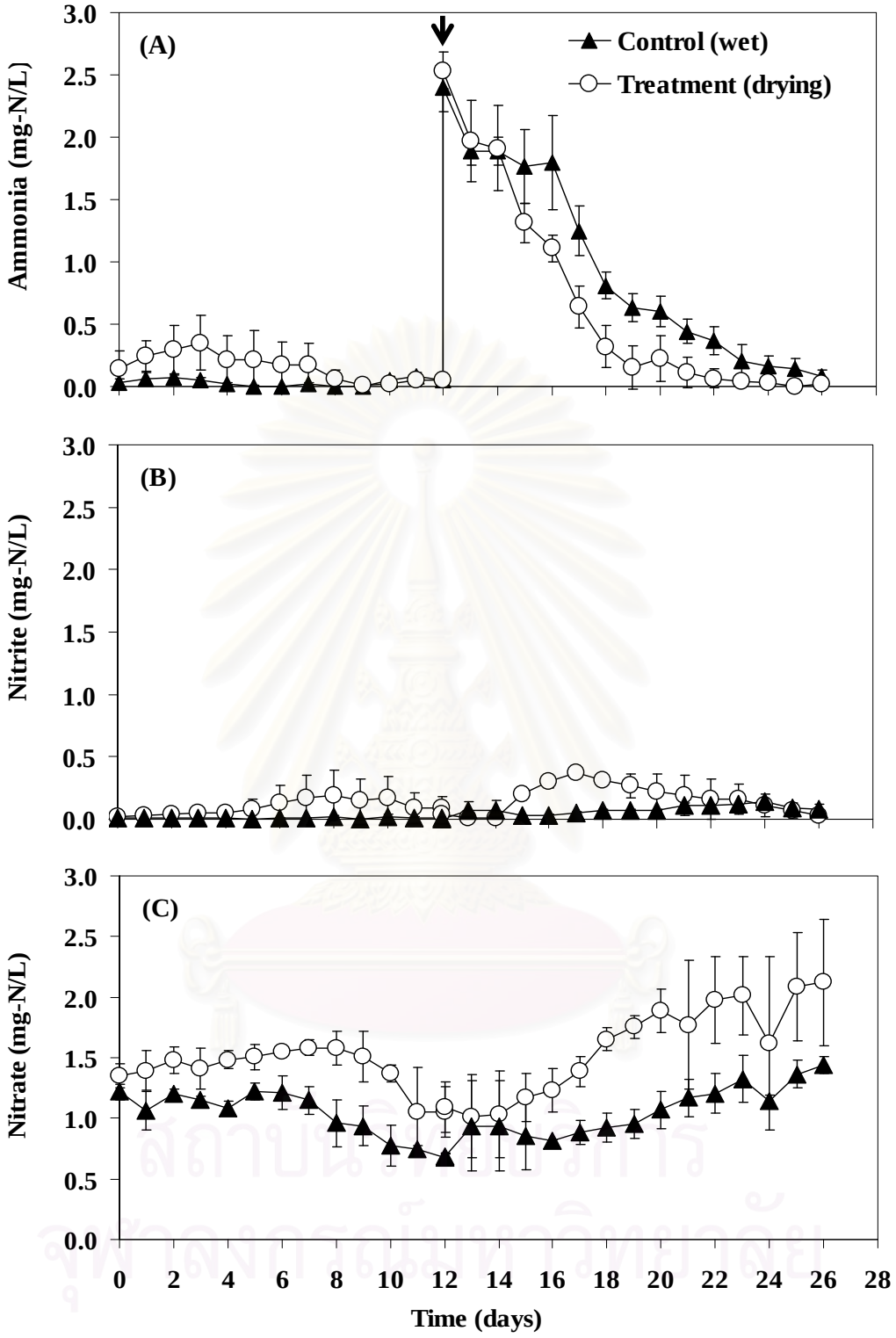


Figure 5.11 Changes of ammonia (A), nitrite (B) and nitrate (C) in water column during 26 day incubation. Control tanks contained wet sediment from shrimp pond while treatment tanks contained sun-dried sediment. The arrow in the graph indicates an addition of ammonium chloride solution in day 12.

In addition, calculation of ammonia removal rate of artificial shrimp pond between day 12 and day 26 in Figure 5.11 was carried out using the following equation (Aslan and Kapdan, 2006);

$$V = \frac{S_0 - S_t}{T_o - T_t}$$

Where V represents the ammonia removal rate (mg-N/L/day), S_0 is the initial ammonia concentration (mg-N/L), S_t is the corresponding ammonia concentration at time T_t (day).

Thereafter, relationship between ammonia concentration (S) and ammonia removal rate (V) was plotted. In theory, variation in ammonia removal rate was depend on ammonia concentration and can be calculated by the following equation (Aslan and Kapdan, 2006):

$$V_{cal} = \frac{V_{max} S}{K_s + S}$$

Where V_{max} is the maximum ammonia removal rate (mg-N/L/day); S is the ammonia concentration (mg-N/L), and K_s is the half saturation constant (mg-N/L).

Calculation of the maximum ammonia removal rate (V_{max}) and the coefficient (K_s) were calculated after plotting between S and S/V where V_{max} is 1/slope and $-K_s$ is the intercept of X-axis. Figure 5.12 and 5.13 shows that V_{max} of control tank was 1.00 mg-N/L/day and of treatment tank was 1.19 mg-N/L/day. The K_s was 1.87 and 1.23 mg-N/L for the control and treatment tanks, respectively.

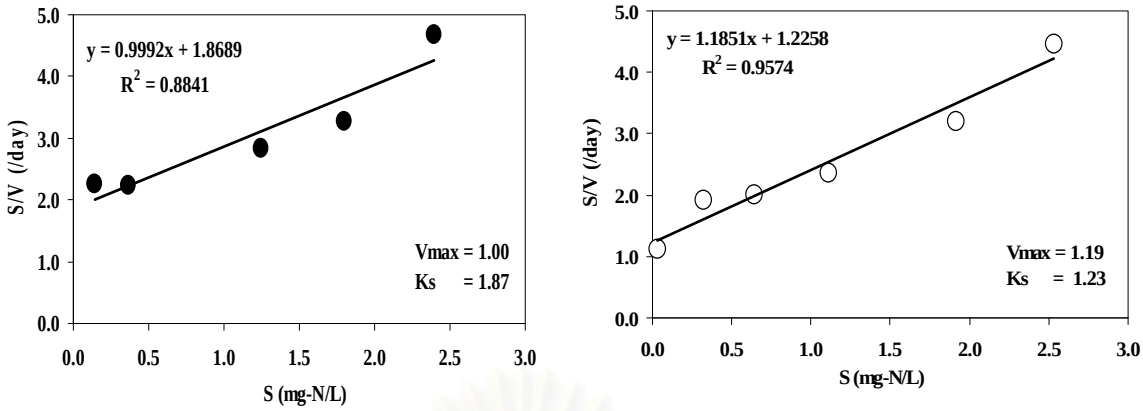


Figure 5.12 Plotting of S versus S/V control (untreated wet sediment) and treatment (sun-dried sediment) outdoor artificial shrimp pond during day 12 to 26. S was concentration of ammonia and V was ammonia removal rate.

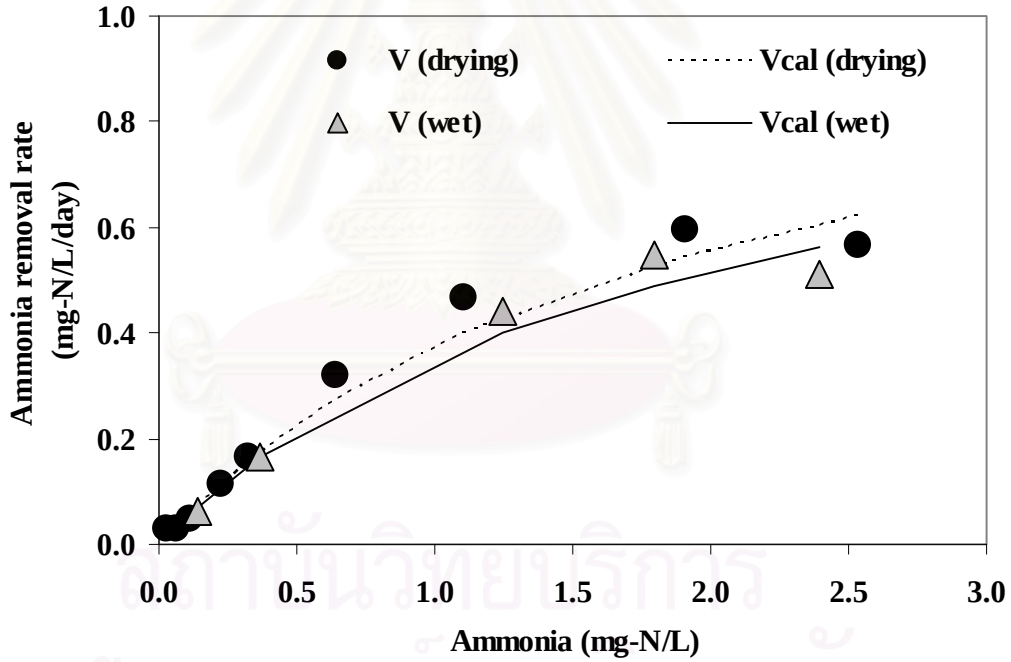


Figure 5.13 Relationship between ammonia concentration and ammonia removal rate of control (untreated sediment) and treatment (sun-dried sediment) tanks as calculated from the data during day 12 to day 26.

Because the experiment was performed under natural condition with sunlight, changes of phytoplankton represented by either cell count and chlorophyll concentration were evaluated. It was found that phytoplankton during the initial day was undetected and the water was very clear. Density of phytoplankton represented by chlorophyll-a, b and c is shown in Figure 5.14. Phytoplankton cells were detected after day 8 with the density between 0.3-6.2x10^4 cells/mL and microscope observation revealed that dominant phytoplankton was the unicellular pennate diatoms (Figure 5.15).

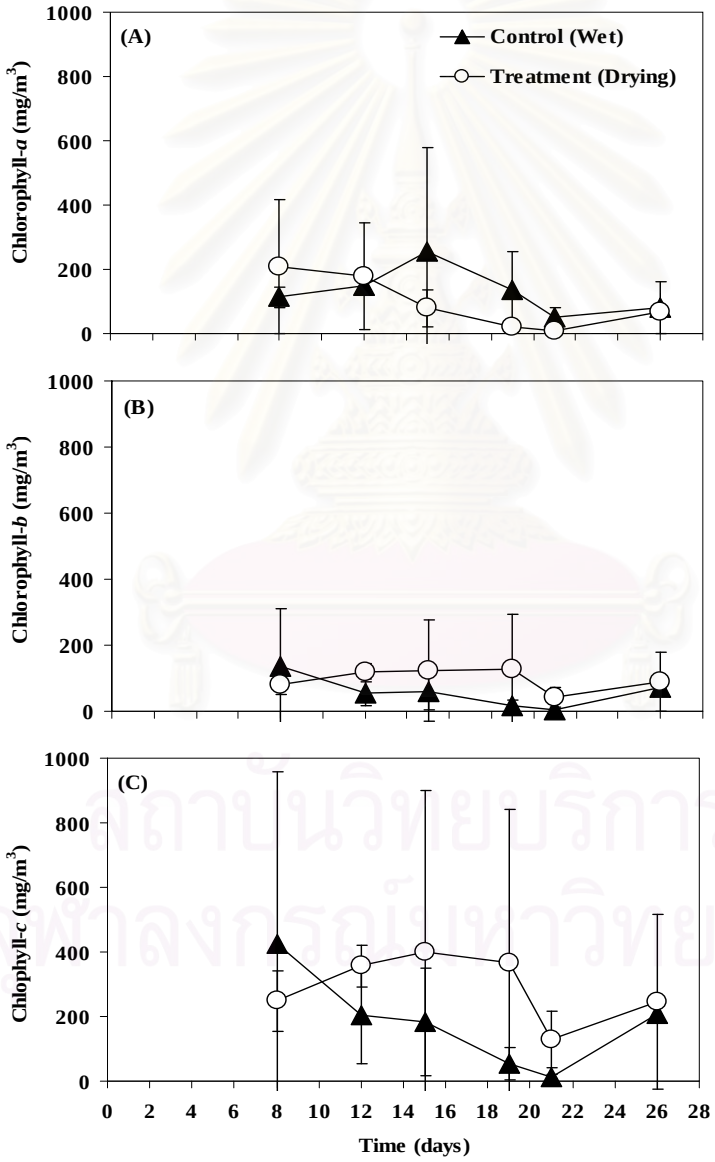


Figure 5.14 Changes in chlorophyll-*a* (A), *b* (B) and *c* (C) in control (untreated wet sediment) and treatment (sun-dried sediment) artificial shrimp pond during 26 days of experiment.

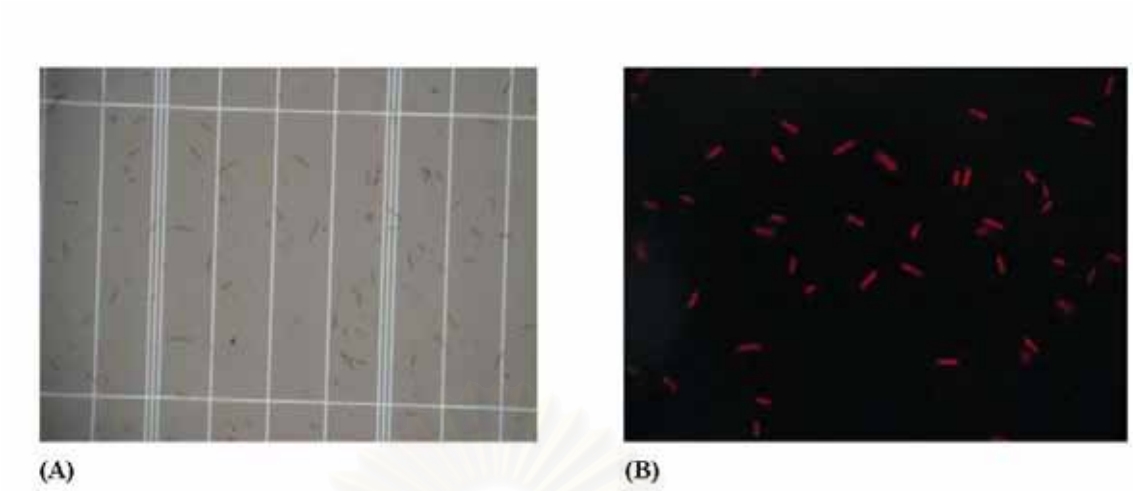


Figure 5.15 Morphology of the dominant diatom under light microscope (A) and fluorescence microscope (B). (Photographed with 10X objective lens)

Total nitrogen and organic matter in sediment at day 0, 12 and 28 were shown in Figure 5.16-5.17. It was found that, after 28 days, total nitrogen in control was constant while total nitrogen in treatment tank was decreased from 0.15 to 0.09 mg-N/g dried sediment but this was not significant (t-test, $P \leq 0.05$). Organic content in sediment of control and treatment tanks was between 18-20%.

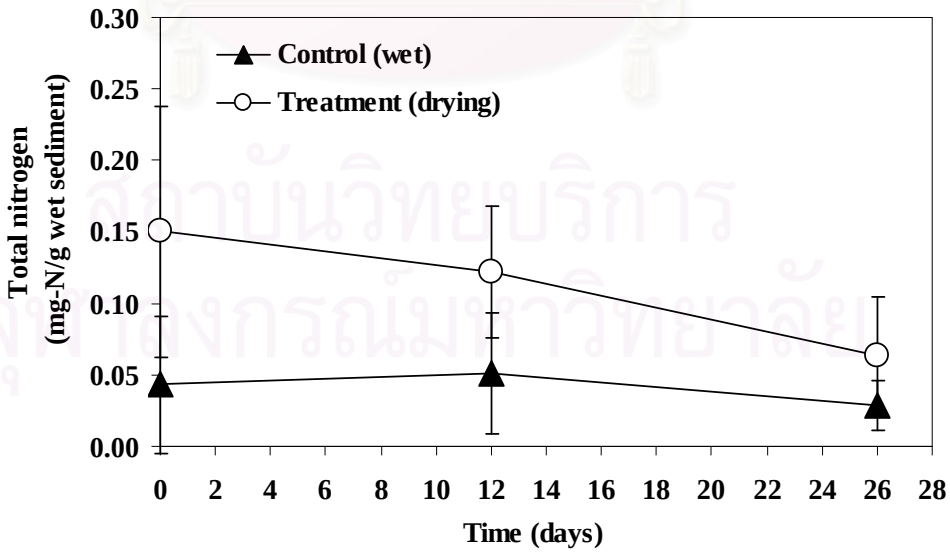


Figure 5.16 Changes in total nitrogen in sediment along the experimental period for 26 days.

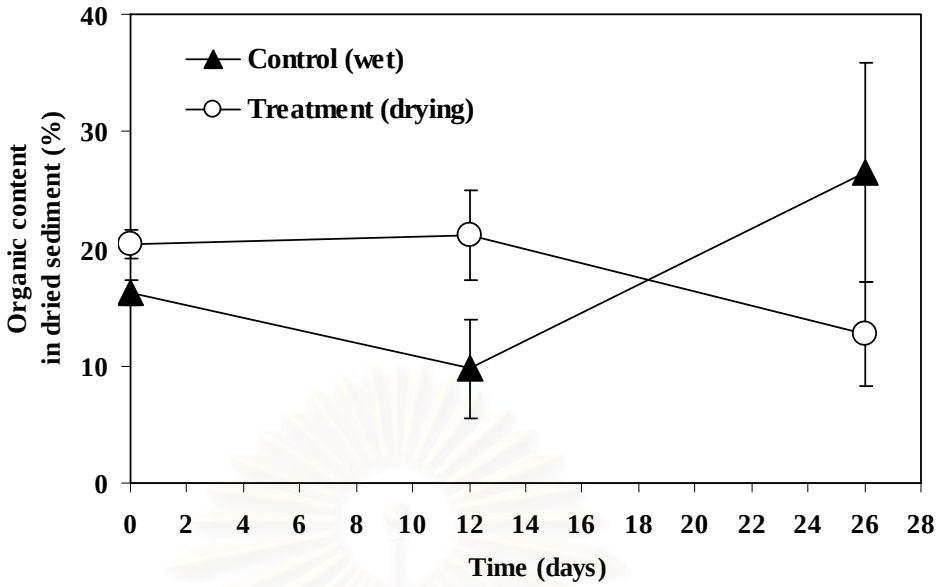


Figure 5.17 Changes in organic carbon in sediment along 26 days of experiment.

5.3.4.3 Microbial diversity

5.3.4.3.1 Bacterial diversity

At days 0, 12 and 26, the bacterial diversity in water and sediment (Figure 5.18) was revealed by PCR-DGGE of 16S rRNA gene fragments obtained from untreated wet sediment (control) and sun-dried sediment (treatment). At the initial day, the DNA banding patterns from control and treatment sediment samples were similar. DGGE profile at day 12 showed that bacteria in control and treatment tanks were obviously difference in number of species and band intensity. The dominant band OUT-3 was not appeared in control tank while the band OUT-5 was particularly appeared in this tank. After 26 days experiment, both OUT-3 and OUT-5 bands were disappeared from DGGE gel but new DNA bands OUT-4 and OUT-6 were found.

The DGGE analysis of bacteria in water column is shown in Figure 5.18. It was found that, four intense DNA bands were appeared both control and treatment tanks at the initial day. Bands OUT-1 and OUT-4 was regularly detected along 26 days of experiment. At day 26, number of intense DNA bands increased in both control and treatment tanks. There were two DNA bands, OUT-1

and OUT-2 that found only in the water column while bands OUT-3, OUT-4 and OUT-6 were found in both water and sediment.

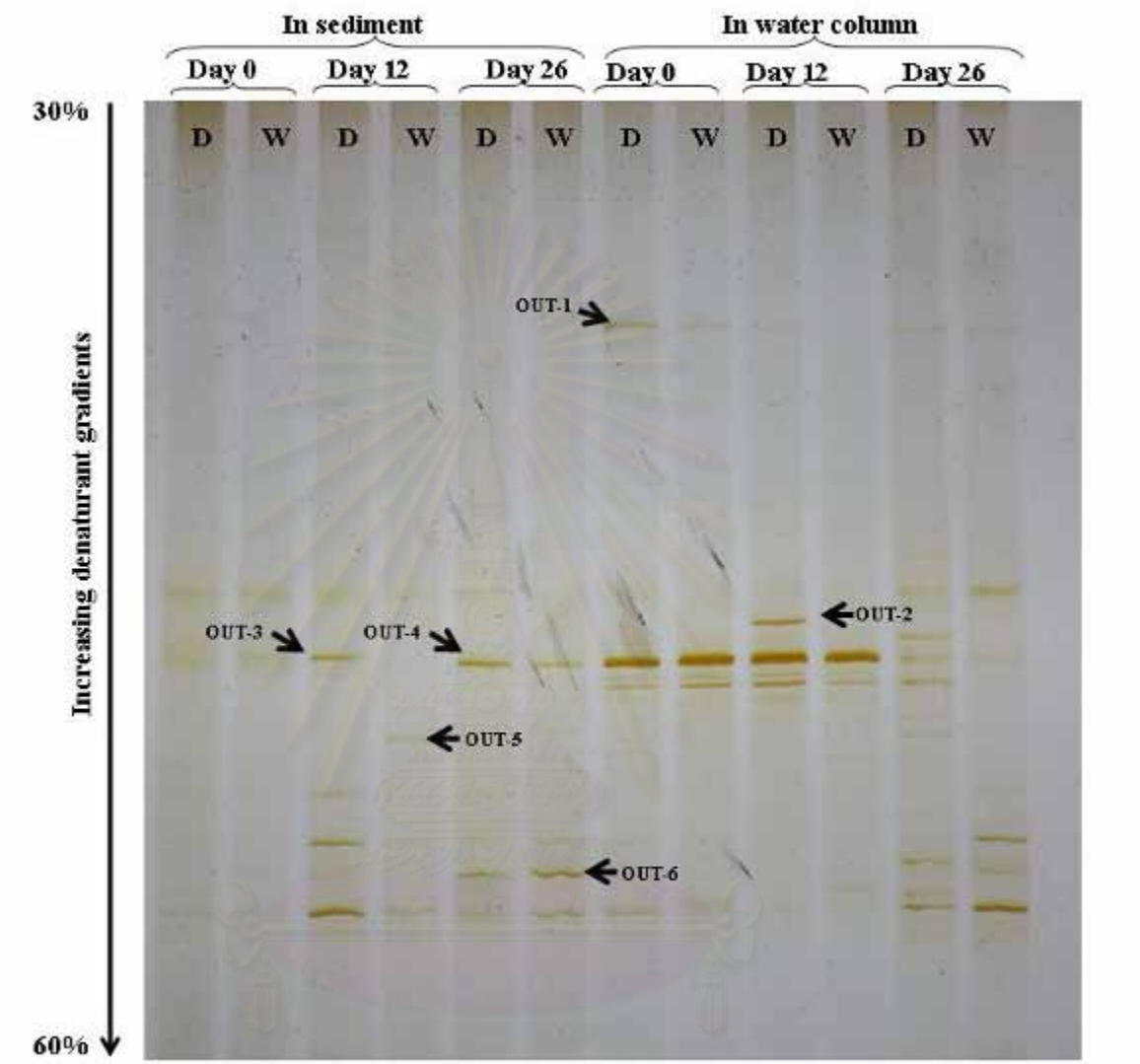


Figure 5.18 DGGE profiles of 16S rDNA genes derived from sediment and water from outdoor artificial shrimp pond during 26 days of experimental period. Lanes D was samples from treatment tank with sun-dried sediment while W was samples from control tank with untreated wet sediment. DNA bands labeled with numbers were then re-amplified and sequenced.

With the UPGMA analysis, a dendrogram was generated using Nei and Li's homology coefficient at 95% of confidence. In Figure 5.19A, DGGE profiles of bacteria in sediment could be separated into two main clusters. The first cluster contained sediment from both control and treatment tanks at the initial day and from treatment tank at day 12. The similarity of bacterial diversity of the first cluster was 100%. Bacterial diversity found in sediment from treatment tank in day 12 and control tank in day 26 was classified into the second cluster. This finding suggested that bacterial diversity was clearly changed over time since the similarity between first and second clusters was lower than 55%.

Figure 5.19B shows the cluster analysis of DGGE band profiles of bacteria in the water which could be separated into two clusters with 68% similarity. The results indicated that bacterial diversity in the water at the initial day was similar in both control and treatment tanks (100% similarity). However, bacteria profile in treatment tank at day 26 was differed to those found in other water samples.

The species richness, evenness and Shannon-Weaver index of bacteria in sediment and water column of control and treatment tanks were evaluated in Figure 5.20. It was found that increase in all indices was observed in sediment from control and treatment tanks while it was not observed in water column.

Six selected DNA bands in Figure 5.18 were excised from DGGE gel and sequenced. DNA sequences were then compared with the NCBI database using BLASTN and compared with RDP II database using Seqmatch program. The related sequences are listed in Tables 5.6 and phylogenetic trees are shown in Figure 5.21. It was found that OUT-1 and OUT-2 were belonged to the Betaproteobacteria. Bands OUT-3 and OUT-5 were related to *Pseudidiomarina* and *Marinobacter* (Gammaproteobacteria), respectively. On the other hand, DNA band OUT-4 was belonged to chloroplast of the unicellular diatom *Nitzschia closterium*.

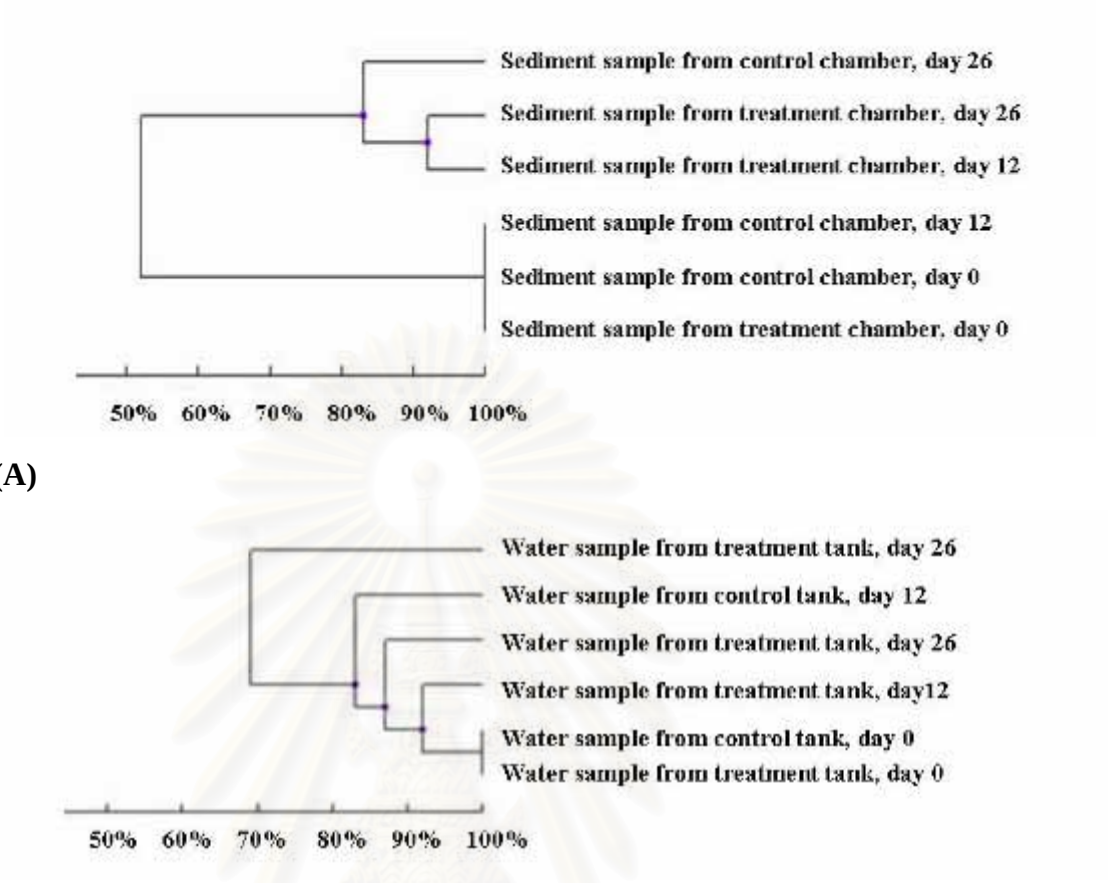


Figure 5.19 The UPGMA dendrogram showing the clustering analyses of DGGE profile in sediment (A) and water (B). Scale bar represented indicates similarity of Nei and Li's coefficients with 95% confidence.

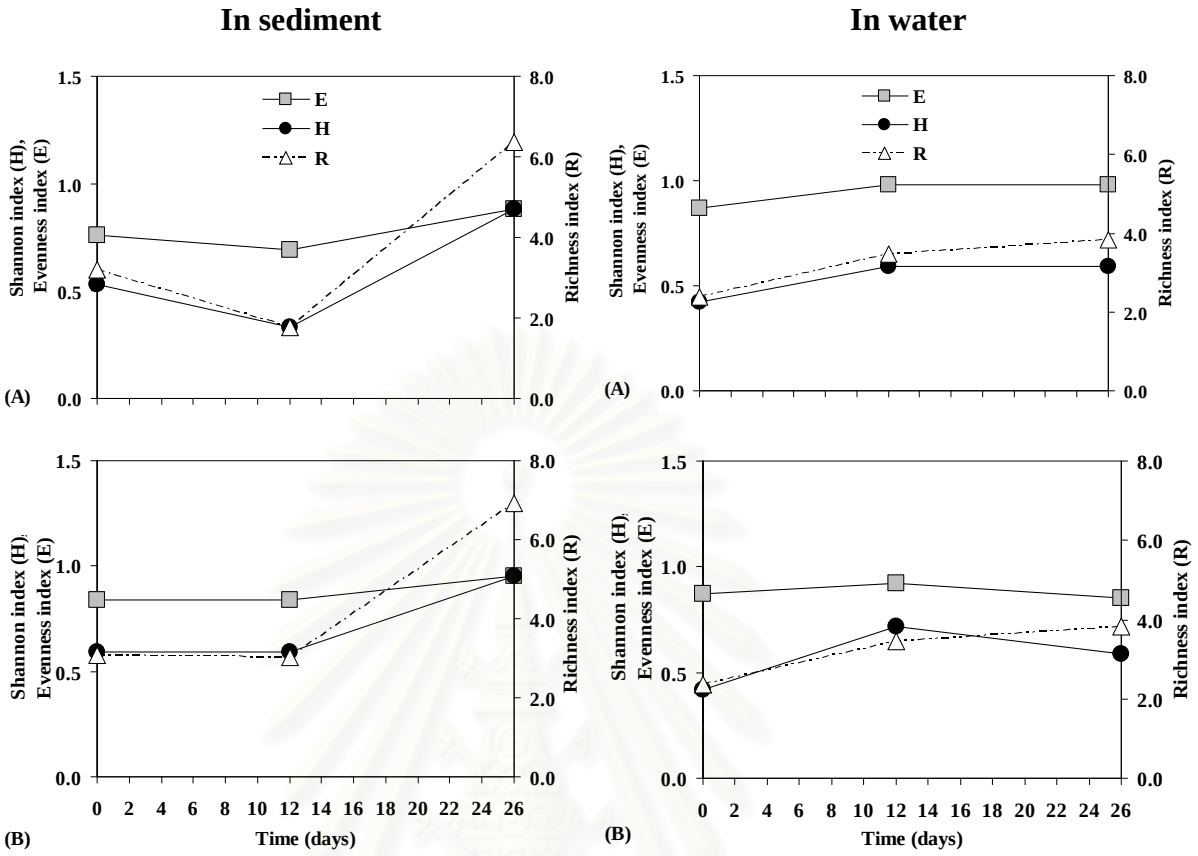


Figure 5.20 Shannon-Weaver index (*H*), species richness (*R*) and evenness (*E*) index in sediment chambers that calculated from DGGE banding patterns in Figure 5.18. A: in control tank packed with untreated wet sediment; B: in treatment tank packed with sun-dried sediment.

Table 5.6 16S rDNA sequence similarity of sequences from selected bands from Figure 5.18 and related bacterial group from RDP II and NCBI data base.

DNA band	RDP II			NCBI		
	ID	Sequence description	Identities (%)	ID	Sequence description	Identities (%)
OUT-1	U70704	clone OM43	60	FJ460107.1	Proteobacterium MS-M-86	100
OUT-2	AB021374	*Burkholderia glathei*	57	EF066726.1	Uncultured betaproteobacterium clone LiUU-30-443	90
OUT-3	AB021371	Str. MBIC1298 ATCC 27123.	69	EU600202.2	*Pseudidiomarina s*p. 8C	100
OUT-4	AJ007876	clone LD27	81	FJ002221.1	*Nitzschia closterium* isolate C14	99
OUT-5	AF148811	*Marinobacter articus*	88	DQ533496.1	Uncultured *Marinobacter* sp. clone 1-24	100
OUT-6	AF170735	Str. QSSC1-18	71	AM92390.1	Flavobacteriaceae bacterium enrichment culture clone DH-1659	100

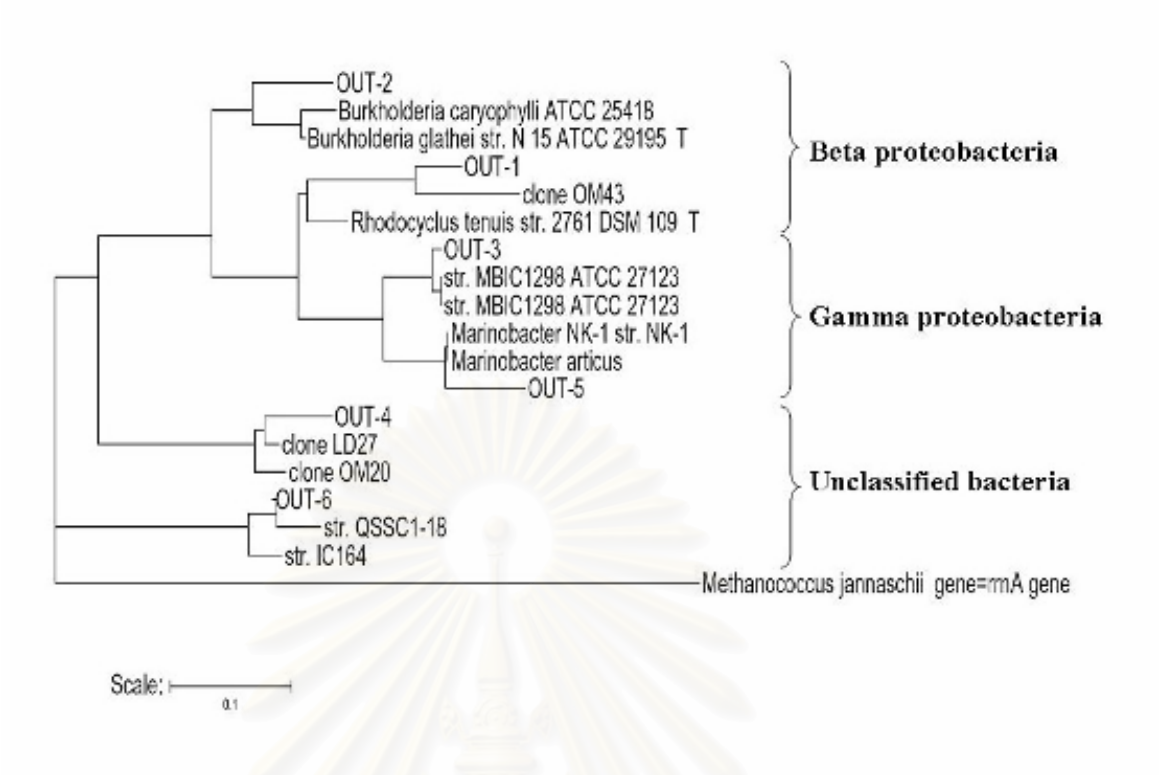


Figure 5.21 Phylogenetic relationship of 16S rDNA fragments obtained from artificial shrimp pond (Figure 5.18). The tree was constructed by using the neighbor-joining method of a 8.1 software in RDP II. Methanogenic archaea (*Methanococcus jannaschi*) was used as an out-group. The bar represents 0.1 nucleotide substitution per position. Bacteria were clustered from the RDP II database.

5.3.4.3.2 Betaproteobacteria AOB diversity

The community of beta-subdivision ammonia oxidizing bacteria (AOB) in the water column and the sediment from the experimental tanks was determined. It was found that PCR product of 465 bp DNA fragments were obtained only from sediment samples at day 28 while it could not be detected in other water and sediment samples at day 0 and day 12. The DGGE results of 16S rRNA genes from Betaproteobacterial AOB in sediment in day 26 are shown in Figure 5.22. It was found that there were two intense DNA bands with similar Rf position in both control and treatment tanks. Sequence analysis of the most intensive DNA band, as indicated by an arrow in Figure 5.22, revealed that it was affiliated with the genera *Nitrosomonas* with the GenBank accession number AF386753.1 (97% similarity). Sequence alignment with RDP ID database also belonged to *Nitrosomonas marina* with 80% similarity.

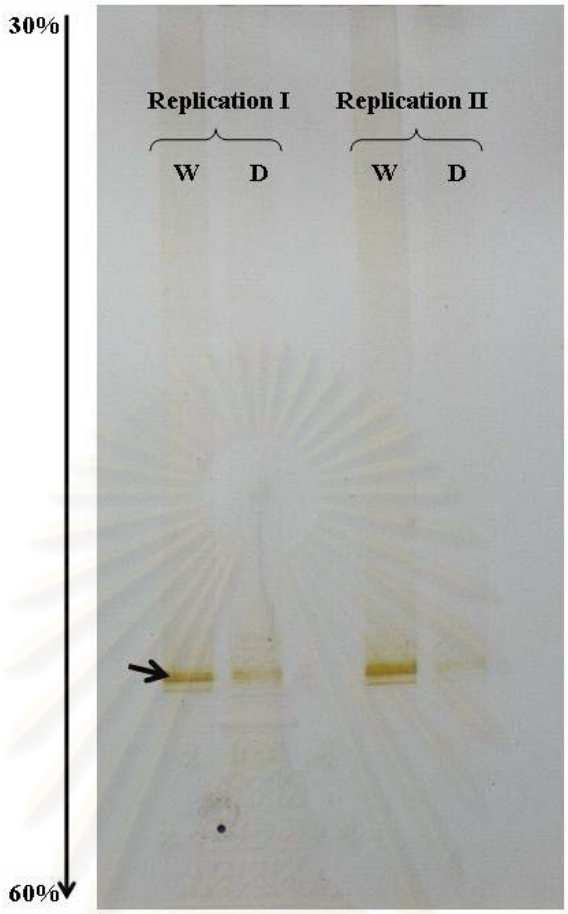


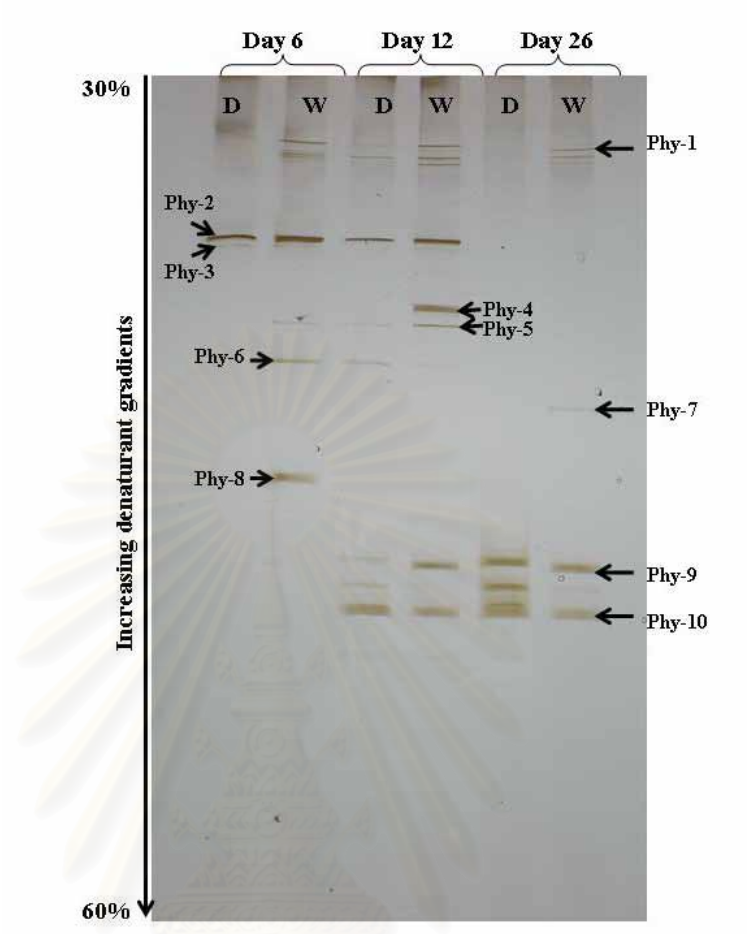
Figure 5.22 PCR-DGGE analysis of 16S rRNA gene amplified with CTO primer sets. The gel was analyzed in duplicate. Lane A and C were sediment from the treatment tanks packed with sun-dried sediment at day 26. Land B and D were from control tanks packed with untreated wet sediment at day 26. An arrow indicates the DNA band to be further re-amplified and sequenced.

5.3.3.3.3 Eukaryotic plankton diversity

Figure 5.23A shows that several bands of PCR products (250 bp) were clearly separated by DGGE. It was found that, during day 6, control tank contained more plankton species (7 bands) than treatment tank which had only 3 bands. New plankton species as illustrated by an apparent of new DNA bands were found in days 12 and 26. Cluster analysis of the DGGE banding patterns from control and treatment tanks revealed that eukaryotic community structure in treatment tank at day 12 was differ from all other samples with the similarity of 54% (Figure 5.23B). Figure 5.24 shows that the evenness (*E*) and Shannon-Weaver index (*H*) in the treatment tank increased while those in control tank decreased after 26 days of the experiment.

With DGGE, the total of 10 DNA bands (Phy-1 to Phy-10) indicated by arrows in Figure 5.23A were excised for sequence analysis. The results are shown in Table 5.7 and the phylogenetic tree is in Figure 5.25. It was found that three major eukaryotic clusters were Bacillariophyta, Arthropoda and unclassified organism. Only Phy-1 could be classified to species (a diatom: *Bacillaria paxillifer*) while other sequences were unnamed or unclassified organisms.

(A)



(B)

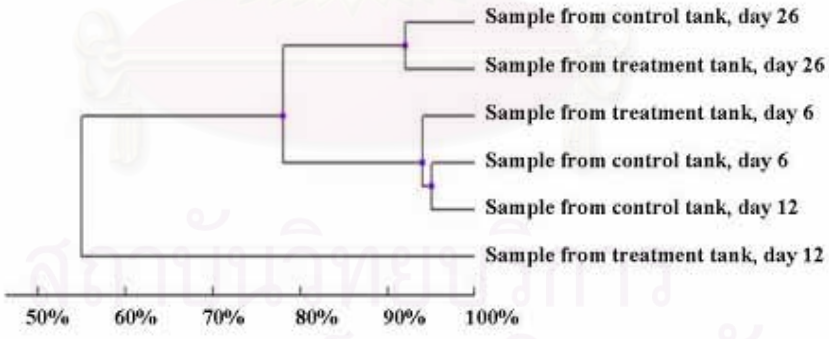


Figure 5.23 (A) 18S rRNA gene was amplified from eukaryotic phytoplankton in outdoor artificial shrimp pond. D represented samples from the treatment tank packed with sun-dried sediment and W represented samples from the control tank packed with untreated wet sediment. (B) UPGMA dendrogram of DGGE banding patterns of eukaryotic microorganisms in the experimental tank. Scale bas represents percentage of similarity coefficient.

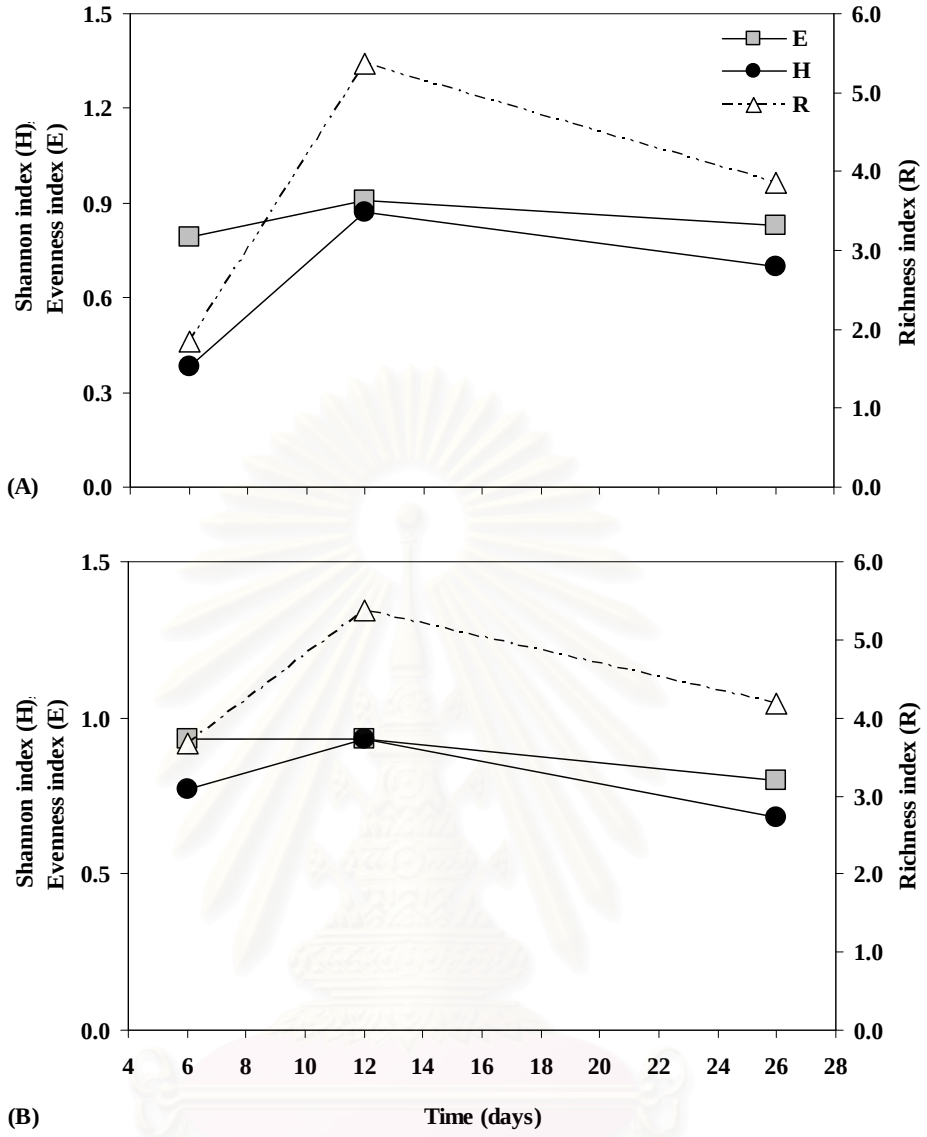


Figure 5.24 Species evenness (*E*), richness (*R*) and Shannon-Weaver diversity (*H*) indices obtained from PCR-DGGE analysis of eukaryotic phytoplankton in Figure 5.23. A = treatment tank packed with sun-dried sediment; B = control tank packed with untreated wet sediment.

Table 5.7 18S rDNA sequence similarities to closest relatives of DNA recovered from the selected bands in the DGGE gel (Figure 5.23)

DNA band	RDP II			NCBI		
	ID	Sequence description	Identities (%)	ID	Sequence description	Identities (%)
Phy-1	Bacl.paxil	*Bacillaria paxillifer*	44	AY256245.1	Uncultured eukaryote isolate E12	84
Phy-2	915Subsa	Unnamed organism	80	EF193000.1	*Attheya septentrionalis*	96
Phy-3	250Tric2	Unnamed organism	91	EU090031.1	*Nitzschia* sp. AnM0026	98
Phy-4	535Brevi	Unnamed organism	48	EU011930.1	*Salpingoca pyxidium* stain ATCC 50929	94
Phy-5	527Insig	Unnamed organism	68	AY916637.21	Uncultured eukaryote clone dpeuk5	99
Phy-6	978Terre	Unnamed organism	59	AY605211.1	Uncultured cercozoan clone Sey076	92
Phy-7	528Elong	Unnamed organism	46	EF628893.1	Uncultured fungus clone T3_IV_3a_21	97
Phy-8	89HVando	Unnamed organism	90	L81944.1	*Heterocypris* sp.	98
Phy-9	Prk.margn	*Prokelisia marginata*	62	AY149194.1	Uncultured eukaryote DGGE band H12	100
Phy-10	87HNatan	Unnamed organism	60	EU380303.1	*Ameira scotti*	96

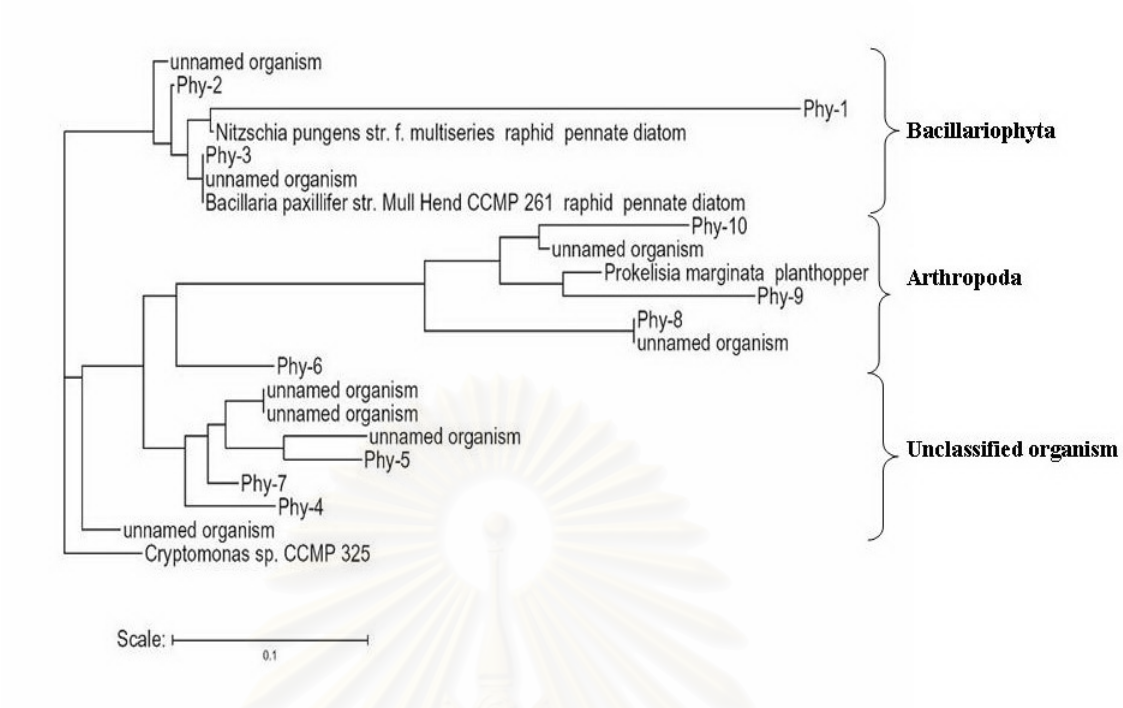


Figure 5.25 The phylogenetic tree of eukaryotic organisms in the experiment tanks, based on the partial sequence of 18S rDNA genes resolved by DGGE. The distance-matrix consensus tree was calculated using the neighbour-joining method with bootstrapping. The 18S rDNA sequence of *Cryptomomas* sp. CCMP 325 (green algae) was used as an out-group species. The bar represents 0.1 nucleotide substitution per nucleotide in 18S rDNA sequences.

5.3.5 Efficiency of ammonia removal rate of outdoor artificial shrimp pond

This section was an addition experiment to evaluate the carrying capacity of an outdoor artificial shrimp pond based on ammonia removal capability. With this experiment, ammonia was added into the tank at approximately 2 mg-N/L and ammonia addition was repeated everyday. Thereafter, decrease in ammonia concentration based on daily basis was calculated as an ammonia removal rate. This experiment was followed up the experiment in section 5.3.4 and operated in the same tanks. Hence, sediment in the artificial shrimp ponds, both control (untreated wet sediment) and treatment (sun-dried sediment), had been acclimated for 26 days (during an experiment in section 5.3.4) before starting this experiment.

Because of long acclimation period during previous experiment (section 5.3.4), ammonia removal rates of artificial shrimp ponds containing untreated sediment or sun-dried sediment were almost resemble (Figure 5.26). It was found that an addition of 0.5 mg-N/L/day ammonia was somewhat higher than the removal capacity of the pond system. As a result, accumulation of ammonia and nitrite was detected (Figure 5.26A-C). In fact, this ammonia loading was a simulation of ammonia excretion by 20 g shrimp cultured at 40 shrimp/m^2. On the other hand, ammonia accumulation was not found when an ammonia loading was reduced to 0.2 mg-N/L/day which equal to ammonia excretion by 20 g shrimp at 16 shrimp/m^2.

With this experiment, ammonia removal rates in both control and treatment tanks were varied between 0.12 to 2.6 mg-N/L/day. Higher ammonia removal rate 0.53-0.61 g-N/m^2/day was obtained with 0.5 mg-N/L/day of ammonia loading. Reduction of ammonia loading to 0.2 mg-N/L/day decreased the average ammonia removal rate to 0.21-0.28 g-N/m^2/day (Table 5.8).

As seen in Figure 5.27, bloom of phytoplankton as indicated by chlorophyll concentration was found with high ammonia addition (0.5 mg-N/L/day). The dominant chlorophyll species was chlorophyll-*c* hence the dominant phytoplankton was the diatoms. However, chlorophyll in artificial shrimp pond supplied with low ammonia (0.2 mg-N/L/day) was significantly low throughout the experimental period.

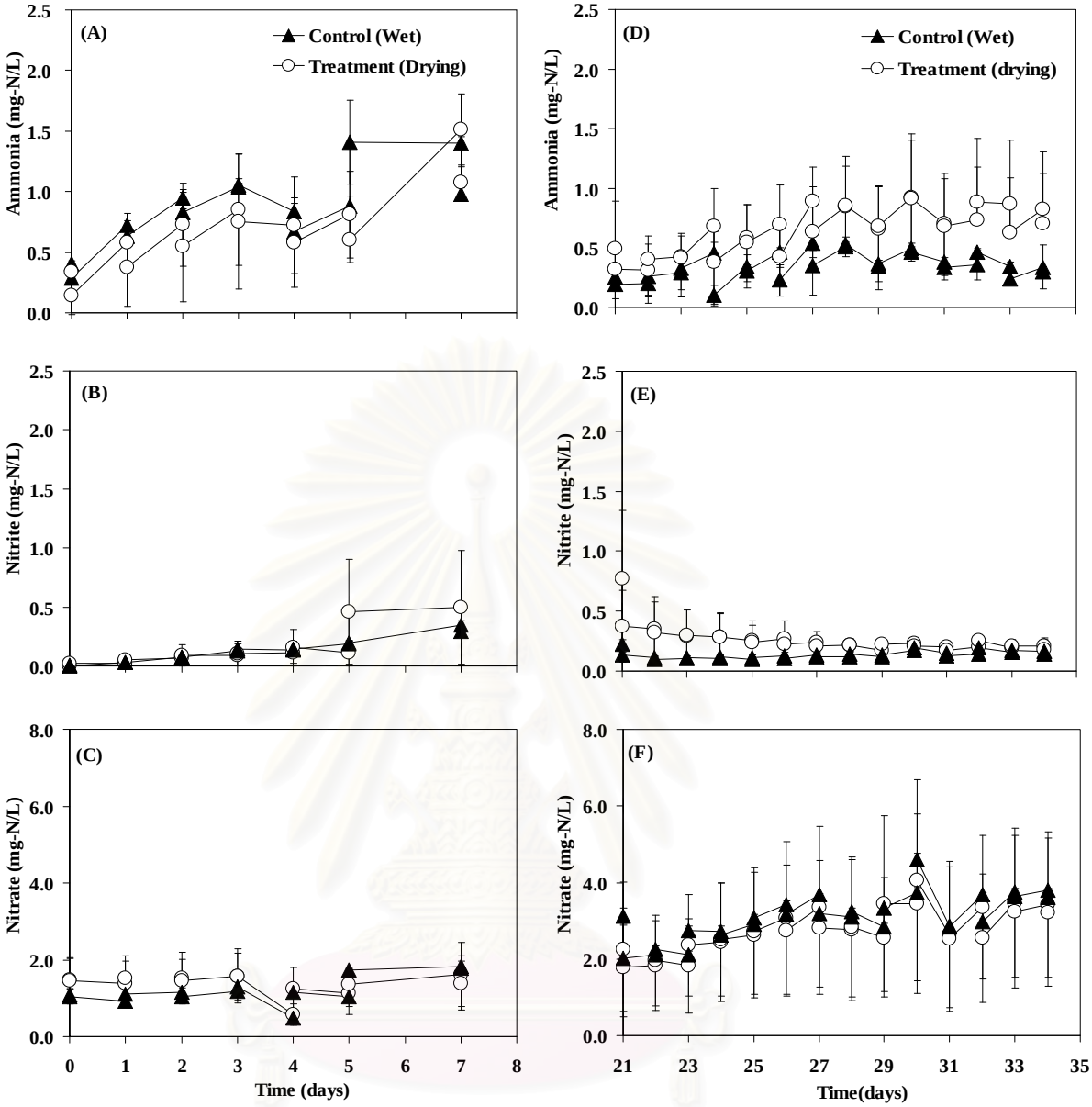


Figure 5.26 Changes of ammonia, nitrite and nitrate in the artificial shrimp ponds. Ammonia-nitrogen was added into tanks at 0.5 mg-N/L/day between day 0 and 7 (A-C) and at 0.2 mg-N/L/day between day 21 and 34 of the experiment (D-F). Control tank was packed with untreated wet sediment and treatment tank was sun-dried sediment.

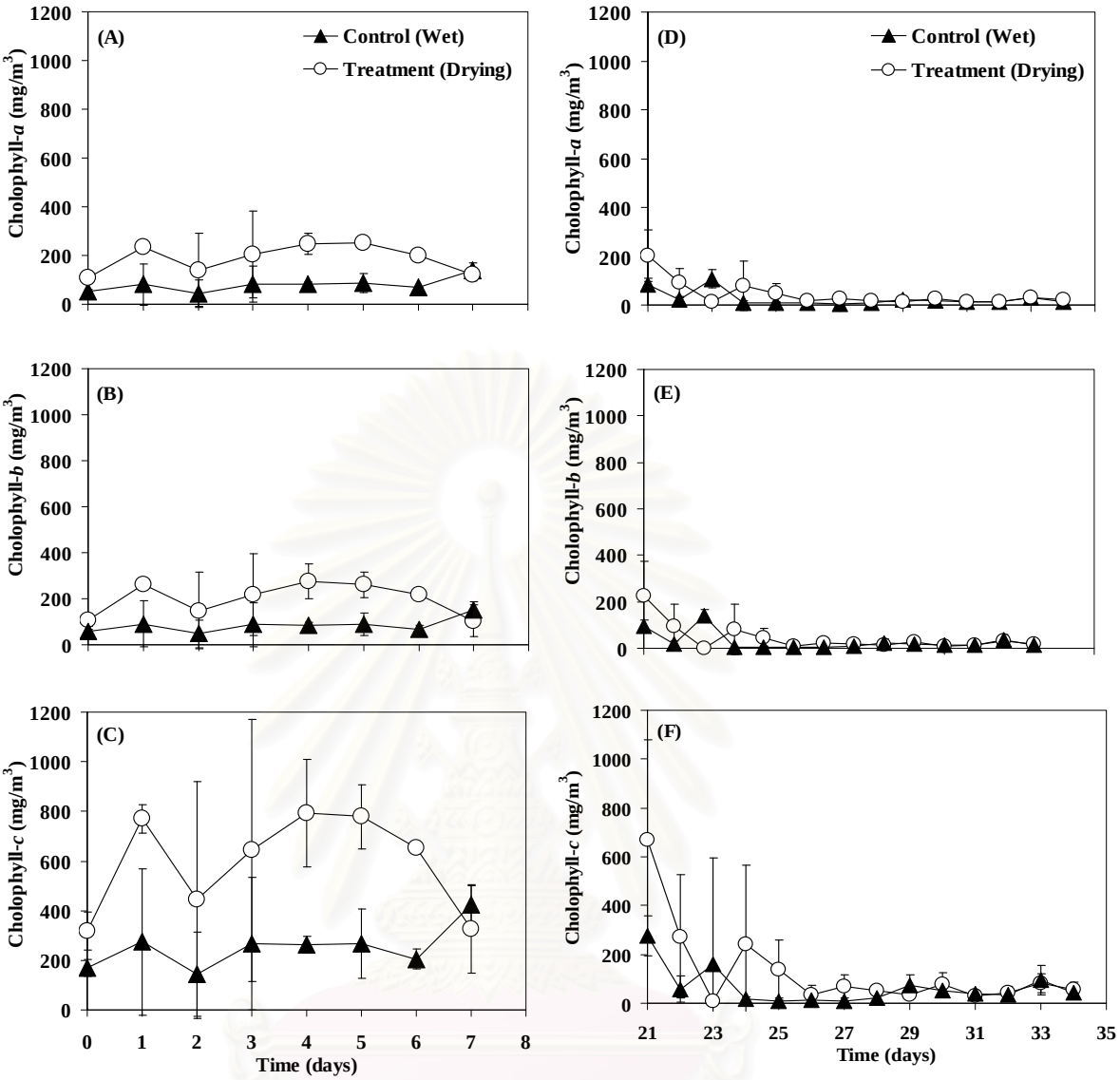


Figure 5.27 Changes of chlorophyll-*a*, *b* and *c* in the artificial shrimp ponds supplemented with ammonia at 0.5 mg-N/L/day (A-C) and at 0.2 mg-N/L/day (D-F). Control tank was packed with untreated wet sediment and treatment tank was sun-dried sediment.

Table 5.8 Mean (±SD) of ammonia removal rate of the artificial shrimp ponds supplemented with ammonia-nitrogen at 0.5 and 0.2 mg-N/L/day. The control tanks contained untreated wet sediment while the treatment tanks contained sun-dried sediment.

Ammonia loading (mg-N/L/day)	Ammonia removal rate					
	(mg-N/L/day)		(g-N/tank/day)		(g-N/m^2/day)	
	Control	Treatment	Control	Treatment	Control	Treatment
0.5	1.03±0.86	0.89±0.31	0.46±0.39	0.40±0.14	0.61±0.51	0.53±0.18
0.2	0.39±0.42	0.52±0.49	0.16±0.18	0.21±0.23	0.21±0.24	0.28±0.29

5.4 Discussions

5.4.1. Effects of dried pond bottom soil on inorganic nitrogen compound conversion and bacterial diversity under laboratory conditions

5.4.1.1 Inorganic nitrogen conversion

The experiments were carried out in sediment chamber under dark condition. Here, activity of phytoplankton was eliminated while most of nitrogen dynamic in the chamber was from bacterial activity. Figure 5.2A revealed that ammonia released immediately after filling seawater to all sediment chambers. It was due to decomposition or ammonification process of high organic content in sediment by many heterotrophic microorganisms *i.e.* bacteria and fungi. The product of ammonification is ammonia or ammonium ion (de Boer and Kowalchuk, 2001). In shrimp pond, a large organic matter is always retained over the pond bottom soil; here it is decomposed and gently released ammonia into the water column (Foy and Rosell, 1991; Hargreaves, 1998; González-Félix *et al*., 2007). So, ammonification is an important regulating process that affects the inorganic nitrogen concentration in the water column. In Figure 5.2A, ammonia concentration in water was highest in treatment-2 chamber which was packed with dried sediment which was exposed with natural sunlight for one week. This could be summarized that sun-dried soil did not

inhibit microbial decomposition process of organic matter. Many researches reported that the decomposition of soil organic matter was found after water filling in the dried pond (Pesaro *et al*., 2004; Wu and Brookes, 2005; Gordon *et al*., 2008). Moreover, Boyd (1995) suggested that change in physical factors including pH, moisture, nutrient and oxygen of soil is affect by nitrogen transformation. With the natural processes, ammonia was eliminated from all experimental units within a week.

After ammonia oxidation in all experimental chambers, increase of nitrite concentration was observed (Figure 5.2B). With dried soil (treatment-1 and 2 chambers), concentration of the nitrite peak was lower than that found with untreated wet soil (control chamber). It was possible that untreated soil contained higher organic content (Figure 5.5). So, ammonia was gradually released to water and it was rapidly converted to nitrite. However, the elimination of nitrite in treatment-1 and in treatment-2 chambers was occurred in day 18 while in control chamber was in day 25 (Figure 5.2B). In theory, nitrite is intermediate in nitrification and in denitrification process. Of these, there are two possible explanations. First, it is possible that incomplete nitrification was occurred. High ammonia concentration is substrate inhibition of nitrite oxidizing bacteria (NOB). Weland *et al*. (1998) reported that NOB is inhibited by ammonia in the range of 0.1-1.0 mg-N/L while ammonia oxidizing bacteria (AOB) is in the range of 10-150 mg-N/L. In addition, activity of NOB is directly correlated with cells density and its strains (Feray and Montuelle, 2002). Incomplete nitrification which resulting in an accumulation of nitrite is one of the common problem in the nitrogen removal systems and in environments including activated sludge, wastewater reservoirs, rivers and aquaculture ponds (Hwang *et al*., 2000; Hargreaves, 1998). Second, it is possible that high nitrite concentration was also inhibited the activity of denitrifying bacteria. As water column was supplied with the near-saturated DO (Table 5.2), this was suitable condition that can promoted activity of aerobic bacteria including autotrophic nitrifying bacteria. In contrast, oxygen is absence from the sediment layer at about 1 to 2 mm in depth so the activity of anaerobic bacteria including heterotrophic denitrification is promoted (Dodds and Jones, 1987; McCaig *et al.*, 1999). Many studies revealed that nitrite accumulation could be found as the resulted from incomplete denitrification due to the presence of oxygen (Aboutboul *et al*. 1995; Barak and van Rijn, 2000).

In Figure 5.2C, constant nitrate concentration over times was found in control and treatment-1 chamber. This was possibly due to the equality of nitrification in aerobic zone and denitrification in anoxic zone. It was confirmed by the ORP values in sediment that was between -60.7 to -157.0 mV (Table 5.2) which was the ORP of denitrification process (Zhang *et al*., 2005). In treatment-2, nitrate was accumulated to high concentration (3.3 mg-N/L) which was unusual for typical sediment chamber. Hence, denitrification could also be influenced by sun-dried process.

In addition, decrease of total alkalinity in the water was found in all experimental chambers (Table 5.2). There were several processes in both aerobic and anoxic condition such as ammonification, nitrification, denitrification, Mn (IV)-, Fe (III)- or SO_4-reductions that relate with alkalinity (Abril and Frankignolle, 2001). In nitrification process, alkalinity is the major carbon source for nitrifying bacteria and is consumed during nitrification. Reduction of alkalinity is evidence that confirmed the occurrence of nitrification in all experimental chambers.

In Figure 5.3, conversion of ammonia, nitrite and nitrate in pore water and extracted sediment was revealed. In pore water, ammonia, nitrite and nitrate was higher than in extracted sediment. It was due to organic matter in the sediments decomposes to ammonia. Thereafter, it is gradually released into pore water and also diffused to water column (Boyd, 1995; Hargreaves, 1998). With natural processes such as precipitation, adsorption, reduction, oxidation, biodegradation and biological uptake, the concentration of nutrients in pore water is high (Tuominen *et al*., 1999). Drying sediment resulted in increase of ammonia in pore water (Figure 5.3B), it indicated that drying soil can be increase oxygen in pore water and organic matter in sediment may be oxidized by aerobic bacteria. Decrease of nitrate in pore water found in all chambers after incubated for 28 days (Figure 5.3C). However, analysis of total oxidized nitrogen in the sediment showed that it was slightly decreased in all experimental units (Figure 5.4). It was probably resulted from nitrogen loss by denitrification process (Franzluebbers *et al*., 1994; Welander *et al*., 1998).

5.4.1.2 Bacterial diversity

At present, denaturing gradient gel electrophoresis (DGGE) is routinely used to assess the diversity of microorganism community and to monitor their dynamics (Muyzer *et al*., 1993; McCaig *et al*., 1999; Jie *et al*., 2008). With DGGE results, bacterial community in untreated, air-dried and sun-dried sediment was different (Figure 5.6). High diversity of bacteria in pond bottom soil (0-2 cm depth) was also detected. Principally, bacteria with aerobic, facultative and anaerobic capability could be simultaneously found in the sediment (Torsvik *et al*., 2002; Rittmann, 2006). Regardless, drying sediment resulted in the loss of number of dominant bacterial species, diversity of bacteria in dried soil under ambient air was slightly changed (Figure 5.6-7). This is possible that decrease of water content in air-dried sediment from 32.3 to 13.2% resulted in bacterial osmotic stress by decrease the substrate diffusion leading to change in their metabolisms (Wu and Brooks, 2005; Fierer and Schimel, 2002). Lost of dominant bands was DES-2, 5 and 7 that is *Bacillus* sp., *Nitrosomonas* sp. and *Methylophaga* sp., respectively (Table 5.3). After drying sediment with a natural sunlight for a week, it was found that water content decreased from 32.3 to 4.8% and at least three DNA bands such as DES-5, 6 and 7 were disappeared. The DES-6 was closely related to *Methylomonas* sp. Nimrat *et al*. (2008) reported that pathogenic bacteria *Vibrio* was eliminated and *Pseudomonas* was decreased by drying of soil with sunlight. In addition, they reported that the addition of probiotic given the high efficiency of organic degradation and pathogenic bacteria removal. In contrast, our result indicated that drying soil can be eliminated both pathogenic bacteria and also beneficial bacteria. Four of sediment bacteria including *Bacillus* sp., *Nitrosomonas* sp., *Methylophaga* sp. and *Methylomonas* sp were eliminated by drying sediment; all of them are effective bacteria for natural waste treatment processes in aquaculture pond. *Bacillu*s is the majority group of probiotic and it can improve water quality by decompose the organic materials and reduce ammonia, nitrite and nitrate (Fast and Menasveta, 2000; Lalloo *et al*., 2008). Nitrifying bacteria *Nitrosomonas*, which commonly found in soil, sewage, freshwater, brackish and seawater (Focht and Verstraete, 1977; Francis *et al*., 2005; Cafferey *et al*., 2007), is an important bacteria in the nitrogen cycle due to it can oxidize ammonia to nitrite. *Nitrosomonas* and other ammonia oxidizing bacteria are essential for nitrogen treatment and other bioremediation processes (Campos *et al.*, 2002). Many

researches evaluated that *Nitrosomonas* sp. had a major role in ammonia removal from aquaculture pond such as a commercial prawn pond (Shan and Abbord, 2001), a marine fish farm (McCaig *et al*., 1999), a closed Tilapia tank (Sesuk *et al*., 2009). An aerobic halophilic methylotrophs *Methylophaga* and *Metylomonas* are widely distributed in soils, mud, and water environments. They can oxidize methane to CO_2 and have a capability of reducing nitrate to nitrite and use ammonia as theirs nitrogen source (Bourne *et al*., 2004).

Water quality and bacterial diversity was also changed with incubation period (Figure 5.2, 5.6 and 5.7). High nitrite accumulation in control chamber was revealed at day 14, here *Bacillus horti*, *Bacillus* sp., *Methylomonas*, *Methylophaga marina* disappeared. In addition, decrease of species richness was found (Figure 5.8). This was possible that their activities are inhibited by high nitrite concentration. After incubated for 28 days, toxic nitrogen substances (ammonia and nitrite) were treated from all sediment chambers due to the activities of natural bacteria. Regarding to a pathogenic bacteria *Vibrio* sp. (DES-10) was disappeared in all sediment but it was appeared after incubated all experimental chambers over two weeks. Of these, it was due to the non-sterilized seawater that will be contaminated this pathogenic bacteria from environments.

The bacterial phylogenetic tree was evaluated that most dominant bacteria in the sediment from shrimp pond was closest to class Proteobacteria (DES-5 through 8 and DES-10). This result agrees with previous study that Proteobacteria is the most abundant in aquatic environments and it is also a major group in shrimp ponds (Muyzer and Smalla, 1998; Sakami *et al*., 2008). In general, heterotrophic bacteria in environments belong to the Proteobacteria and those takes an important role in nutrients cycle, especially carbon and nitrogen cycle (Prosser, 1986).

5.4.2 Effects of pond bottom soil drying on ammonia release and removal under laboratory conditions

With the results in section 5.3.2, ammonia and nitrite released from sediment were removed from the sediment chamber. However, this removal process required more than three weeks to eliminate these nitrogenous wastes. Drying

sediment under ambient air in the dark and under direct sunlight for one week did not delay the ammonia removal but even accelerated the removal processes. In general, ammonia is gradually produced from sediment and continuously diffused to the overlying water. Ammonia released is simultaneously occurred with other biogeochemical processes of nutrients such as sedimentation, absorption, mineralization, nitrification and denitrification (Ignatieva, 1999). So, evaluation of ammonia removal process in high organic content sediment can not be done accurately by a simple model. For example, accumulation of ammonia in the water was results from several processes but it can be revealed that ammonia producing was higher than ammonia removal processes. Unfortunately, identification of each involved process was still unclear.

Adding ammonia chloride solution into the experimental chambers could accelerated the activity of sediment bacteria. With control chamber packed with wet sediment, it was found that ammonia was rapidly decreased from 2 mg-N/L to below 0.1 mg-N/L within one week (Figure 5.10A). This was suggested that ammonia is an important substrate to stimulated activities of natural AOB in aquatic environments. Similar result was found in Qiu and McComb (1996) in which an increase of ammonium concentration in lake sediment stimulated nitrification process of the sediment. In contrast, ammonia in treatment chamber with dried sediment was gradually increased from 2 to 5 mg-N/L for 5 days after ammonia addition. Perhaps, drying soil can induced the oxidation of organic compounds that produced ammonia. Goedon *et al*. (2008) found that drying soil could increase the concentration of dissolved inorganic nitrogen in sheep-grazed grasslands, Lancashire, UK.

Base on our finding, the wet sediment that added with 2 mg-N/L and non added with ammonium chloride solution, nitrite was eliminated after 7 days of incubation. High nitrite accumulation was detected in both treatment chambers that supplied and unsupplied with ammonium chloride whereas nitrite was not detected after 24 days incubation (Figure 5.10B).

Figure 5.10B clearly illustrated that soil drying resulted in an inhibition of soil microbial activities especially with nitrification process. As a result, incomplete nitrification with nitrite accumulation up to 6 mg-N/L was found in treatment chambers. Kaplan *et al*. (2000) suggested that the observed dynamics of nitrite accumulation in environments could be described by various processes as

follows: incomplete denitrification that is blocked at the nitrite stage under aerobic condition, inhibition of nitrite oxidation under aerobic condition and a sudden increase in the rate of ammonia oxidation without a parallel increase in subsequent nitrite oxidation rate. In addition, lost of nitrate in treatment chambers was detected at day 14 of incubation, at the same time, the highest of nitrite accumulation was found between 5 to 6 mg-N/L. This was due to the incomplete nitrification process in which the activity of NOB was inhibited. Thereafter, nitrite was completely oxidized to nitrate after day 26. In general, there are two major sources of nitrate in the soil denitrification, first, the diffusion of nitrate from the overlying water and, second, mineralization of ammonia to nitrate by nitrification (Nielsen *et al*., 1996).

5.4.3 Changes of inorganic nitrogen compound conversion and microorganism diversity in an outdoor artificial shrimp pond

5.4.3.1 Nitrogen conversion in an outdoor artificial shrimp pond

The results from this study revealed that sun drying could inhibit nitrification process of the sediment in treatment tank. Hence, accumulation of ammonia and nitrite was higher than that found in control tank with untreated sediment (Figure. 5.11A-C). These results were similar to the trend of nitrogen conversion which was evaluated under laboratory conditions (see in section 5.4.1-2). Therefore, dried soil stimulated the decomposition of soil organic matter but inhibited nitrification process.

Pesaro *et al*. (2004) reported that the decomposition of soil organic matter increases following the filling water of dried soil because several parameters in soil such as pH, moisture and nutrients were changed. Nitrogen conversion was appeared rapidly after adding seawater into the tanks. The first findings was ammonia releasing by ammonification process and followed by ammonia oxidation to nitrite, thereafter nitrite concentration was decreased but without nitrate accumulation. This was due to the coupling of nitrification and denitrification in the water column and in sediment layer. However, the toxic nitrogen compounds including ammonia and nitrite was eliminated within 12 days. By the way, the highest concentration of ammonia, nitrite and nitrate in the outdoor

treatment tanks was lower than that found in the treatment-2 chambers under laboratory condition (section 5.3.2). This was due to the volume of water and the surface area of sediment in the experimental chamber and in an outdoor thank was different (Table 5.9). Moreover, it was possible that some of nitrogen was assimilated by phytoplankton cells (van Rijin *et al*., 1986). In general, the growth of diatom is also high in the environment that is an organically rich (Hargreaves, 2006). As the concentration of chlorophyll-*c* was higher than chlorophyll-*a* and *b*, hence, diatom was the dominant species in the outdoor artificial shrimp pond (Figure 5.14).

Table 5.9 Comparison of conditions and inorganic nitrogen compounds in the treatment sediment chambers under dark laboratory condition (section 5.3.2) and in treatment artificial shrimp pond under outdoor condition (section 5.3.4). Both were packed with sun-dried sediment.

Experimental unit	Condition	Maximum Ammonia (mg-N/L)	Maximum Nitrite (mg-N/L)	Maximum Nitrate (mg-N/L)	Working volume (L)	Sediment surface area (m^2)	V/A ratio
Sediment chamber	Dark condition	1.69 (day 2)	2.06 (day 11)	3.64 (day 21)	1.4	0.01	140
Artificial shrimp pond	Outdoor condition	0.35 (day 3)	0.19 (day 8)	1.98 (day 8)	450	0.73	616.4

Remark: V/A is the ratio of water volume (L) to sediment surface area (m^2) of the experimental units.

After the total elimination of ammonia and nitrite in day 12, ammonium chloride was applied into the control and treatment artificial shrimp pond as the simulation of shrimp excretion. It was found that the similar trend of nitrogen conversion was occurred in control and in treatment tanks (Figure 5.11A-C). Therefore, the first part of the experiment before ammonia addition was also an acclimation for nitrifying bacteria to grow and activated. After that 2 weeks acclimation period, bacteria in sun-dried sediment could be recovered and became active to the new environment. This was confirmed by an almost equal rate of

ammonia removal in control and in treatment tank after ammonia addition (Figure 5.12-13). Like in the aquatic environment, the natural nitrogen conversion processes such as sedimentation, ammonia release, nitrification and denitrification can be varied with different environments (Ignatieva, 1999; Jones *et al.*, 1995).

5.4.3.2 Microorganisms diversity in an outdoor artificial shrimp pond

It was found that duration and method of soil treatment affected bacterial community in the outdoor artificial shrimp pond (Figure 5.18-20). In general, change of the bacterial diversity is depended on the environmental factors including biological, chemical and physical factors (Torsvik *et al.*, 1996). Bacterial diversity indexes in the water of both control and treatment outdoor artificial shrimp ponds were substantially low (Figure 5.20). Apart of the light-sensitive bacterial species, number of suspended bacteria in the water was much lower than the bacteria deposited at the pond bottom. On the other hand, highly diverse of bacterial species was found in sediment that was rich with organic carbon and nutrients including nitrogen compounds.

In water column, marine pennate diatom *Nitzschia clodterium* was detected as the dominant species. With the 16S rRNA gene amplification, it has always found the 16S rRNA gene from chloroplasts of microalgae especially nanoplankton and also picoplankton in aquatic environments (Risgaard-Petersen *et al.*, 2004; Crosbie *et al.*, 2003). In addition, diatom can be aggregated as the diatom mats on the sediment surface if light is presented. Many benthic diatoms also have a capability to grow under heterotrophic condition in the dark. In general, diatoms have a significant impact on chemical components that related to the metabolism of other microorganisms and nutrients conversion (Risgaard-Petersen *et al.*, 2004). For example, activity of AOB could be suppressed by the faster growth benthic diatoms which have higher nitrogen uptake rates (Risgaard-Petersen *et al.*, 2004).

The major clusters of bacteria in the experimental tanks were β and also γ-proteobacteria (Figure 5.21). These bacteria usually play an important role of carbon and nitrogen dynamic (Torsvik *et al.*, 1996). Betaproteobacteria AOB has been detected in marine sediments and in water by the amplification of 16S rRNA gene and *amoA* gene (Freitag and Prosser, 2004; Xia *et al.*, 2005). Figure 5.22 revealed the DGGE patterns of 16S rRNA gene fragment amplified from AOB in

sediment at day 26 of the experiment. It was found that the community of AOB in water column throughout the experimental period and also in sediment layer between days 0 through day 12 was not detected. It was indicated that, AOB was mostly distributed at the sediment surface and it was not significantly brought into the water column by water circulation or bottom currents. In addition, *Nitrosomanas* was only one of AOB species that was detected in the untreated wet sediment and in sun-dried sediment (Figure 5.22). Of these, it represented that AOB could recovered to the environments in an outdoor artificial shrimp pond after an acclimation for approximately three weeks. Moreover, growth of AOB and their activity was also stimulated by ammonium chloride addition at day 12 of experiment.

Apart from bacteria, diversity of eukaryotic plankton was also evaluated by amplification of 18S rRNA gene fragments. Figures 5.23A-B revealed the DGGE pattern of eukaryotic plankton and cluster analysis. DNA sequence after DGGE analysis showed that there were three major groups of eukaryotic plankton including Bacillariophyta (diatoms: phytoplankton), Arthropoda (zooplankton) and Unclassified organisms. Qingyun *et al.* (2008) reported that bacterial community in Yangtze River, China was correlated with the variable of nitrate, DO and silicate, whereas, the eukaryotic plankton community was correlated with the concentration of phosphate, DO and silicate. Unlike our results, a constant of DO was found throughout the experimental period and concentration of ammonia, nitrite and nitrate in control tank and in treatment tank was almost similar. Dissimilar of eukaryotic plankton in control and treatment should possible the results from sediment drying.

CHAPTER VI

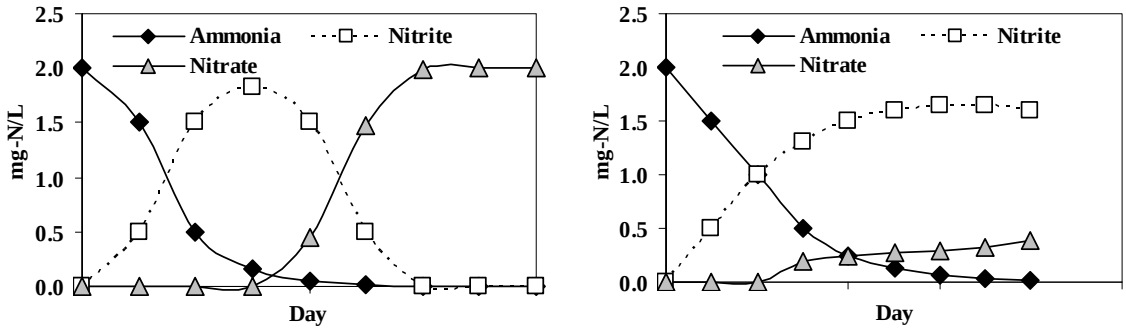
GENERAL DISCUSSION

6.1 Inorganic nitrogen conversion in aquaculture systems

In traditional earthen aquaculture pond, the major nitrogen waste treatment process is performed mainly by microbial activity at the pond bottom soil. On the other hand, treatment processes in the lining pond and the indoor recirculating systems are regularly in the water by suspended-growth of microorganisms or in nitrification biofilter by attached-growth of bacteria, respectively (Moriaty, 1997: Kassila, 2003).

In general, startup the nitrification biofilter in the recirculating aquaculture systems requires at least 30 to 45 days incubation period to fully activate the nitrifying bacteria (Timmons *et al.*, 2002). With this period, ammonia is used as the nitrogen sources for ammonia oxidizing bacteria that convert ammonia to nitrite. Next, nitrite oxidizing bacteria in the biofilter is then convert nitrite to nitrate. In an ordinary acclimation of nitrification biofilter, as illustrated in Figure 6.1, decreased of ammonia is always follow up by a peak of nitrite. Then nitrite is oxidized to nitrate which is the final product of nitrification. At the end of acclimation period, accumulation of nitrate to the concentration that equivalent to the initial ammonia concentration is found. On the other hand, aquaculture system without bottom soil such as the outdoor plastic lining pond has been suffering from nitrite accumulation during the cultivation crop (Figure 6.1). Previous study found that an accumulation of nitrite in outdoor lining pond was due to incomplete nitrification process (Kutako *et al*., 2007).

The results from this study suggested that the pond bottom soil is also need to be acclimated in order to achieve complete nitrification process. This acclimation is similar to the acclimation procedure of biofilter typically used in the indoor recirculating aquaculture systems. After pond acclimation, natural nitrification and denitrification processes in the sediment can be performed simultaneously hence the pond system will be ready for nitrogen loading from aquaculture activities.



Complete nitrification Incomplete nitrification

Figure 6.1 Drawing illustrate the typical inorganic nitrogen conversion during an acclimation period of nitrification biofilters in the indoor recirculating aquaculture system (Left) and nitrite accumulation in outdoor lining pond which is a result of incomplete nitrification (Right).

Pattern of inorganic nitrogen conversion in earthen pond or tank with bottom sediment is different from that found in biofilter acclimation tank. Figure 6.2 was drawn according to the experimental results in both Chapter 4 and 5, in which ammonia was always released from sediment to the water column almost immediately after water filled up. Thereafter, ammonia was oxidized to nitrite hence a peak of nitrite was followed up. Duration needed for ammonia or nitrite elimination was varied due to environmental condition as shown in Table 4.11. Throughout the ammonia and nitrite oxidation processes, nitrate in water column was remained constant or slightly increase. This was due to denitrification process in sediment layer that convert nitrate to nitrogen gas.

After pond acclimation process and nitrifying bacteria in the sediment surface was fully activated, aquaculture pond then ready for nitrogen loading. As shown in Figure 6.2 which redrawn from the result in Chapter 5 section 5.3.3-4, high ammonia concentration is rapidly eliminated within a few days. During ammonia elimination, small peak of nitrite can be found but nitrate concentration is almost constant. This characteristic is always found if nitrogen loading is within the nitrogen carrying capacity of the pond system.

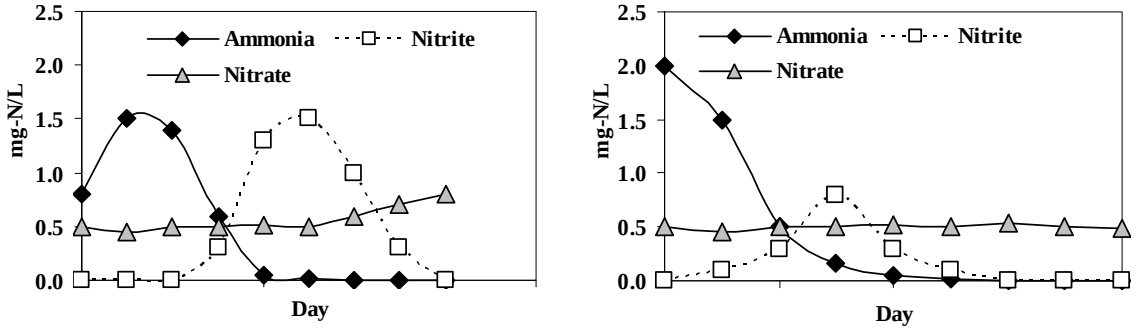


Figure 6.2 Drawing illustrates pattern of inorganic nitrogen conversion in earthen pond. Left drawing represents ammonia and nitrite peaks during pond acclimation period. Right drawing shows the ammonia removal in the pre-acclimated earthen pond.

Acclimated earthen ponds were able to carry out a complete nitrification. The results in section 5.3.5 revealed that ammonia removals in artificial shrimp ponds by sun-dried and untreated sediments were comparable. At the nitrogen loading of 0.5 mg-N/L/day, ammonia removal rates were determined from 0.53 – 0.61 mg N/m^2/day. From this information, it was clear that the corresponding ammonia removal rates were unable to handle nitrogen waste introduced as can be confirmed by excessive ammonia concentrations in water. Ammonia removal rates were determined from 0.21 – 0.28 mg-N/m^2/day after nitrogen loading was reduced to 0.2 mg-N/L/day. Under the new condition, ammonia was completely degraded to nitrate within a day, thus implying that this level of nitrogen loading was within a capacity of earthen ponds to handle. By assuming that about 20 – 30% of feed nitrogen was utilized by shrimps, waste loading at 0.2 mg-N/L/day, and shrimp weight at 20 g, it was possible to carry out the shrimp cultivation as high as 16 shrimps/m^2. Figure 6.3 summarizes the described results.

Therefore, shrimp ponds should be properly prepared to accommodate the occurrence of nitrification and denitrification as means to treat nitrogen wastes. The obtained results are important information that may be used for the development of proper technology to improve waste handling capacity of intensive shrimp production systems.

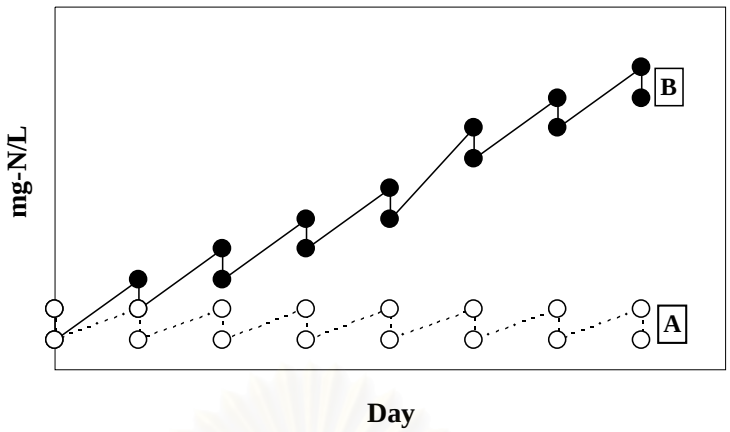


Figure 6.3 Pattern of inorganic nitrogen conversion in earthen ponds. Line A: nitrogen loadings within the carrying capacity. Line B: nitrogen loadings higher than the carry capacity.

6.2 Change in bacterial diversity in pond bottom soil

Many environmental factors including salinity, DO, alkalinity addition, methanol addition, and illumination were able to influence microbial diversity in aquaculture ponds. For instance, denitrifying bacteria were likely to dominate after the methanol addition. Sun-drying of earthen pond, in particular, had pronounced effects on bacteria and planktons. Excluding pathogenic bacteria such as *Vibrio* sp. and *Psuedomonas* sp., beneficial species including probiotic *Bacillus* sp. and *Nitrosomonas* sp. were eliminated after earthen ponds had been exposed to sunlight. *Nitrosomonas* sp. was able to recover nitrifying activity after approximately three weeks of acclimation. Detection of microbial diversity based on the DGGE techniques confirmed that bacterial communities in earthen ponds were dynamic and sensitive to changing environmental conditions.

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The results from this study provide important data to help us understand the inorganic nitrogen conversion and microbial diversity in the sediment from shrimp pond and it can be concluded as following:

1. Rapid change in salinity from 5 to 20 PSU had no effect on ammonia oxidation process but slightly affected nitrite oxidation since higher peak of nitrite was found with 20 PSU sediment chambers. Denitrification was somewhat inhibited due to higher accumulation of nitrate in 20 PSU chambers. In addition, the dominant bacterial species such as *Marinobacter* sp. and *Psuedomonas* sp. disappeared after salinity changed from 5 to 20 PSU.

2. With low DO concentration (2.5 mg-O_2/L), high ammonia (>4.4 mg-N/L) and nitrite (>3.9 mg-N/L) was released to water column due to ammonification process. However, low DO significantly inhibited nitrification process so ammonia in treatment chambers with low DO was eliminated in 8 days which was two days more than in control chambers with high DO (7 mg-O_2/L). Moreover, low DO also slightly decreased the diversity of bacteria in the sediment.

3. Addition of $NaHCO_3$ (at the C:N ratio of 2:1) into the sediment chambers accelerated the nitrogen conversion by ammonia oxidation, nitrite oxidation and denitrification processes. In addition, bacterial diversity in sediment from treatment chambers with $NaHCO_3$ was higher than controls.

4. Addition of methanol at C:N ratio of 2:1 into the sediment chambers prolonged the ammonia and nitrite peaks due to an inhibition of nitrification process. Increase the C:N ratio to 4:1 had higher effect to nitrification and the highest nitrite concentration (>7.9 mg-N/L) was found. Hence it could be summarized that methanol addition has a negative effect on nitrification process in earthen pond. During the experiment, nitrate in water column was almost constant. This indicated that denitrification was simultaneously occurred. Results from DGGE analysis

suggested that denitrifying bacteria *Vibrio* sp., *Psuedomonas* sp., *Planococcus* sp., *Streptomyces* sp. and *Thioalcalovibrio* sp. were stimulated after methanol addition.

5. Conversion of inorganic nitrogen compounds in dark sediment chamber was assumed as a sole bacterial activity while illuminated sediment chamber revealed the activity of both bacteria and phytoplankton. Ammonia removal in light chamber was a combination of ammonia oxidizing by bacteria and ammonia uptake by phytoplankton. However, light could also inhibit nitrification process. This was indicated by an accumulation of ammonia and nitrite at approximately two folds of that found in the dark chambers after organic nitrogen adding. This was even incorporated with phytoplankton bloom in the light chambers. The results suggested that the major role of inorganic nitrogen removal in aquaculture pond was through bacterial nitrification and denitrification processes rather than photosynthetic microorganisms.

6. Decomposition of organic matter in sediment chambers was promoted in the treatments with dried sediment, as described by a peak of ammonia. In sun-dried sediment, nitrite was retained at high concentration (2 mg-N/L) due to inhibition of nitrite oxidation. Moreover, the beneficial bacteria including *Bacillus* sp., *Methylomonas* sp., *Methylophaga* sp. and especially nitrifying bacteria *Nitrosomonas* sp. was disappeared from the sun-dried sediment during the experiment.

7. Sun drying strongly inhibited nitrification process in both ammonia oxidation and nitrite oxidation steps in the sediment chambers. Peaks of ammonia up to 5 mg-N/L and nitrite up to 6 mg-N/L were found in sun-dried sediment chambers but no peak was detected in control chambers with untreated sediment. Ammonia and nitrite in control chambers were totally eliminated in eight days while it took up to three weeks in treatment chambers. Moreover, accumulation of nitrate in treatment chambers revealed that sun-dried sediment also inhibited denitrification process.

8. In an outdoor artificial shrimp pond, sun-dried soil inhibited nitrification process in which peaks of ammonia and nitrite were found in treatment tank. However, the ammonia concentration in artificial shrimp pond was lower than that found in the sediment chamber because of phytoplankton uptake. After 12 days acclimation period, ammonium chloride at 2.5 mg-N/L were added into both tanks. It was found that both control and treatment tanks could eliminate ammonia to below

0.1 mg-N/L in 14 day. Estimation using Michelis-Menten kinetics equation revealed that maximum ammonia removal rate (V_{max}) of the artificial shrimp pond was between 1.00-1.19 mg-N/L/day and K_s was between 1.23-1.87 mg-N/L.

9. In the untreated wet sediment and in sun dried sediment, number of bacterial in the water of the outdoor artificial shrimp pond was substantially low and was not included in DGGE analysis. In contrast, high bacterial diversity was detected in sediment samples. With the ammonia oxidizing bacteria (AOB), *Nitrosomonas* sp. was found in sun-dried sediment tank at day 26. This result illustrated that AOB could recovered to the environments in shrimp pond after acclimation for three weeks. PCR-DGGE analysis also showed that sun-drying clearly damaged both prokaryote and eukaryote microorganisms in the sediment.

10. After pond acclimation until complete nitrification was achieved, simulation of shrimp excretion by repeat addition of ammonium chloride in to the artificial shrimp pond was performed. It was found that ammonia removal efficiency in untreated wet sediment and sun-dried sediment tanks was similar (Figure 5.26). This was summarized that bacteria in sun-dried sediment could recovered and became active to the new environment. With low ammonia loading at 0.2 mg-N/L/day, the average ammonia removal rate in both artificial shrimp ponds was approximately 0.39-0.52 mg-N/L/day that was equal to 0.21-0.28 mg-N/m^2/day. Higher ammonia removal rate 0.89-1.03 mg-N/L/day (or 0.53-0.61 mg-N/m^2/day) was obtained with 0.5 mg-N/L/day but ammonia was retained in shrimp pond at high concentration.

7.2 Recommendations

1. Sediment samples used in each experiment in Chapter 4 were collected with different time. Hence, organic content, nitrogenous waste and other sediment characteristics were somewhat varied in each experiment. This might affect the dissimilarly trend of nitrogen conversion of each experiment.

2. Because some of parameters in the water such as organic matter, total suspended solid and total nitrogen was not detected in this study. Evaluation of these parameters to estimate nutrient flux is recommended for further study.

3. It is difficult to distinguish the efficiency of each biological process that play on nitrogen cycle in earthen pond. Due to the interaction of many process in water and sediment layers such as ammonification, assimilation, nitrification, denitrification and anaerobic ammonia oxidation (anammox) always occurred at the same time. Detail study on each process can be performed using the specific chemical inhibitor to block enzyme or using the specific primers to detect functional gene related with each process.

4. With the results from this study, it was illustrated that soil drying was harmful to some beneficial bacteria especially bacteria that involved with nitrogen conversion. However, these bacteria can be recovered after an appropriate acclimation period of approximately three weeks.

5. It is not only the indoor nitrification biofilter system that need proper acclimation technique to activate nitrifying bacteria, outdoor earthen pond also need approximately 2-3 weeks acclimation before natural nitrification-denitrification processes in the sediment layer can work properly and the pond system then ready to received nitrogen loading from aquaculture activity.

6. Base on natural nitrogen removal process in the artificial shrimp pond condition, the carrying capacity of shrimp culture as simulated by repeat addition of ammonia was approximately 260 g shrimp/m^2 or equal to shrimp stocking density of 26 shrimp/m^2 with 10 g shrimp. At this density, ammonia excretion was totally eliminated within one day. To obtain higher shrimp density, strong aeration and other attempt to reduce nitrogen waste is needed.

REFERENCES

Abed, R. M. M., Kohls, K. and de Beer, D. 2007. Effect of salinity changes on the bacterial diversity, photosynthesis and oxygen consumption of cyanobacterial mats from an intertidal flat of the Arabian Gulf. Environmental Microbiology 9(6): 1384-1392.

Aboutboul, Y., Arbiv, R. and Van Rijn, R. 1995. Anaerobic treatment of fish culture effluents: volatile fatty acid mediated denitrification. Aquaculture 133: 21–32.

Abril, G. and Frankignoulle, M. 2001. Nitrogen-alkalinity interactions in the highly polluted Scheldt basin (Belgium). Water Research 35(3): 844-850.

Acosta-Nassar, M. V., Morell, J. M. and Corredor, J. E. 1994. The nitrogen budget of a tropical semi-intensive freshwater fish culture pond. Journal of World Aquaculture Society 25: 261–270.

Alabaster, J. S., Shurben, D. G. and Knoweles, G., 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon. Journal of Fish Biology. 15: 705–712.

Alonso-Rodrıguez, R. and Paez-Osuna, F. 2003. Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. Aquaculture 216(1-4): 316-336.

APHA, 1992. Standard methods for the examination of water and wastewater. 18th edition, Maryland, USA: American Public Health Association.

Apple, T. 1998. Non-biomass soil organic N: the substrate for N mineralization flushes following soil drying-rewetting and for organic N rendered CaCl2-extractable upon soil drying. Soil Biology & Biochemistry 30: 1445-1456.

Aslan, S. and Kapdan, I. K. 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological engineering 28: 64–70.

Avnimelech, Y. and Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220: 549-567.

Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264: 140–147.

Barak, , Y. and Van Rijn, J. 2000. Relationship between nitrite reduction and active phosphate uptake in the phosphate-accumulating denitrifier *Pseudomonas* sp. strain JR 12. Applied and Environmental Microbiology 66(12): 5236–5240.

Berounsky, V. M. and Nixon, S. W. 1993. Rates of nitrification along an estuarine gradient in Narragansett Bay. Estuaries 16(4): 718-730.

Binnerup, S.J., Jensen, K., Revsbech, N.P., Jensen, M.H. and Sørensen, J. 1992. Denitrification, dissimilatory reduction of nitrate to ammonium, and nitrification in a bioturbated estuarine sediment as measured with ^{15}N and microsensor techniques. Applied and Environmental Microbiology 58: 303–313.

Bissett, A., Bowman, J. and Burke, C. 2006. Bacterial diversity in organically-enriched fish farm sediments. FEMS Microbiology Ecology 55: 48-56.

Bodelier, P. L., Libochant, J. A., Blom, C. W. P. M. and Laanbroek, H. J. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Applied and Environmental Microbiology 62(11): 4100–4107.

Boyd, C. E. 1995. Bottom soils, sediment and pond aquaculture. Chapman&Hall. New York. 348 p.

Boyd, C. E. and Daniels, H. V., 1993. Liming and fertilization of brackishwater shrimp ponds. Journal of Applied Aquaculture 2: 221–234.

Boyd, C. E., Wood, C. W. and Thunjai, T. 2002. Aquaculture ponds bottom soil quality management. PD/A CRSP. Oregon. 41 p.

Bourne, D. G., Young, N., Webster, N., Payne, M., Salmon, M., Demel, S. and Hall, M. 2004. Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, *Panulirus ornatus*. Aquaculture 242: 31–51.

Briggs, M. R. P. and Funge-Smith, S. J. 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. Journal of Fisheries Management 25: 789–811

Brown, J.R., Gowen, R.J. and McLusky, D.S. 1987. The effect of salmon farming on the benthos of a Scottish sea loch. Journal Experimental Marine Biology and Ecology 109: 39-51.

Brown, M. R., Jeffrey, S. W., Volkman, J. K. and Dunstan, G. A., 1997. Nutritional properties of microalgae for mariculture. Aquaculture 151: 315–331.

Burford, M. A, and Longmore, M. R. 2001. High ammonium production from sediments in hypereutrophic shrimp ponds. Marine Ecology Progress Series 224: 187-195.

Burford, M. A. and Lorenzen, K. 2004. Modeling nitrogen dynamics in intensive shrimp ponds: the role of sediment remineralization. Aquaculture 229: 129–145.

Burford, M. A. and Williams, K. C. 2001. The fate of nitrogenous waste from shrimp feeding. Aquaculture 198: 79–93.

Caffrey, J. M., Sloth, N. P., Kaspar, H. and Blackburn, T. H. 1993. Effect of organic loading on nitrification and denitrification in a marine sediment microcosm. FEMS Microbiology Ecology 12: 159–167.

Cafferey, J. M., Bano, N., Kalanetra, K. and Hollibaugh, J. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. The ISME Journal 1: 660–662.

Campos, J. L., Mosquera-Corral, A., Sanchez, M. And Lema, J. M. 2002. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. Water Research 36: 2555–2560.

Cocci, M., Riechmann, D., Bodelier, P. L. E., Stefani, S., Zwart, G. and Laanbroek, H. J. 2005. Effect of salinity on temporal and spatial dynamics of ammonia-oxidizing bacteria from intertidal freshwater sediment. FEMS Microbiology Ecology 53: 359–368.

Colt, J. and Tchobanollus, G., 1978. Chronic exposure of channel catfish, *Ictalurus puntuatus*, to ammonia: effects on growth and survival. Aquaculture 15: 353–372.

Crosbie, N. D., Pockl, M. and Weisse, T. 2003. Rapid establishment of clonal isolates of freshwater autotrophic picoplankton by single-cell and single-colony sorting. Journal of Microbiological Methods 55: 361– 370.

Das, M., Royer, M. V. and Leff, L. G. 2007. Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Applied and Environmental Microbiology 73(3): 756–767.

de Boer, W. and Kowalchuk, G. A. 2001. Nitrification in acid soils: micro-organisms and mechanisms. Soil Biology & Biochemistry 33: 853-866.

Diez, B., Pedrosalio, C., Marsh, T. L. and Massana, R. 2001. Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. Applied and Envirnmental Microbiology 67(7): 2942-2951.

Dodds, W. K. and Jones, R. D. 1987. Potential rates of itrification and denitrification in an oligotrophic freshwater sediment system. Microbial Ecology 14: 91-100.

Dorigo, U., Volatier, L. and Humbert, J-F. 2005. Molecular approaches to the assessment of biodiversity in aquatic microbial communities. Water Research 39: 2207-2218.

Egna, H. S. and Boyd, C. E. 1997. Dynamics of pond aquaculture. CRC press, USA. 437 p.

EPA, 1976. Measurement of photochemical oxidants in the atmosphere. Federal Register 41: 44049-44057.

Fast, A. W. and Menasveta, P. 2000. Some recent issues and innovations in marine shrimp pond culture. Reviews Fish Science 8:151–233.

Fenchel, T., King, G. M. and Blackburn, T. H. 1998. Bacterial biogeochemistry: the ecophysiology of mineral cycling. Academic press. USA. 293 p.

Feray, C. and Montuelle, B. 2003. Chemical and microbial hypotheses explaining the effect of wastewater treatment plant discharges on the nitrifying communities in freshwater sediment. Chemosphere 50: 919-928.

Ferrari, V. C., and Hollibaugh, T. J. 1999. Distribution of microbial assemblages in the central Arctic Ocean basin studied by PCR/DGGE: analysis of a large data set. Hydrobiologia 401:55-68.

Fiere, N. and Schimel, J. P. 2002. Effect of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology & Biochemistry 34: 777-787.

Findlay, D. L., Heck, R. E., Hendze, L. L. Stainton, M. P. and Regehr, G. W. 1994. Relationship between N_2-fixation and heterocyst abundance and its relevance to the nitrogen budget of Lake 227. Canadian Journal of Fisheries and Aquatic Sciences 51: 2254-2266.

Focht, D. D. and Verstraete, W. 1977. Biochemical ecology of nitrification and denitrification. Advance of Microbial Ecology 1: 135-211.

Foy, R.H. and Rosell, R. 1991. Loadings of nitrogen and phosphorus from a Northern Ireland fish farm. Aquaculture 96: 17–30.

Francis, C. A., Roberts, K. J., Bemant, J M., Santoro, A. E. and Oakley, B. B. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102(41): 14683-14688.

Franzluebbers, K., Weaver, R. W., Juo, S.R. and Franzluebbers, A. J. 1994. Carbon and nitrogen mineralization from cowpea plants part decomposing in moist and in repeatedly dried and wetted soil. Soil Biology & Biochemistry 26: 1376-1387.

Freitag, T. E. and Prosser, J. I. 2003. Community structure of ammonia-oxidizing bacteria within anoxic marine sediments. Applied and Environmental Microbiology 69(3): 1359–1371.

Freitag, T. E. and Prosser, J. I., 2004. Differences between betaproteobacterial ammonia-oxidizing communities in marine sediment and those in overlying water. Applied and Environmental Microbiology 70(6): 3789-3793.

Gelfand, I., Barak, Y., Even-Chen, Z., Cytryn, E., Krom, M., Neori, A. and van Rijn, J. 2003. A novel zero-discharge intensive seawater recirculating system for culture of marine fish. Journal of World Aquaculture Society 34: 344-358.

Glass, C. and Silverstein, J. 1999. Denitrification of high nitrate, high salinity wastewater. Water Research 33: 223–229.

González-Félix, M. L., Gómez-Jiménez, S., Perez-Velazquez, M., Allen Davis, D. and Velazco- Rameños, J. G. 2007. Nitrogen budget for a low salinity, zero-water exchange culture system: I. Effect of dietary protein level on the performance of *Litopenaeus vannamei* (Boone). Aquaculture Research 38: 798-808.

Gordon, H., Haygarth, P. M. and Bardgett, R. D. (2008). Drying and rewetting effects on soil microbial community composition and nutrient leaching. Soil Biology & Biochemistry 40: 302-311.

Gowen, R. J. and Bradbury, N. B. 1987. The ecological impact of salmon farming in coastal waters: A review. Oceanography Marine Biology Annuals Review 25: 563-575.

Green, B.W. and Boyd, C.E. 1995. Chemical budgets for organically-fertilized fish ponds in the dry tropics. Journal of World Aquaculture Society 26: 284–296.

Gross, A., Boyd, C. E. and Wood, C. W. 2000. Nitrogen transformations and balance in channel catfish ponds. Aquacultural Engineering 24: 1–14.

Gross, A., Nemirovsky, A., Zilberg, D. and Nejidat, A. 2006. Soil nitrifying enrichments for enhancement of biofiltration systems in intensive recirculating saline water aquaculture. Aquaculture 37(2): 12-14.

Guisasola, A., Petzeta, S., Baezaa, J. A. Carreraa, J. And Lafuente, J. 2006. Inorganic carbon limitations on nitrification: Experimental assessment and modeling. Water Research 41(2): 277-286.

Hamazah, Z. and Ghararah, A. 1996. Biological denitrification of high nitrate water: infuence of type of carbon source and nitrate loading. Journal of Environmental Science and Health 31 (7): 1651-1668.

Hamlin, H. J., Michaels, J. T., Beaulaton, C. M., Graham, W. F., Dutt, W., Steinbach, P., Losordo, T. M., Schrader, K. K. and Main, K. L. 2008. Comparing denitrification rates and carbon sources in commercial scale upflow denitrification biology filters in aquaculture. Aquacultural Engineering 38: 79-92.

Handy, R. D. and Poxton, M. G., 1993. Nitrogen pollution in marine culture: toxicity and excretion of nitrogenous compounds by marine fish. Reviews in Fish Biology and Fisheries 3: 205–241.

Hargreaves, J. A. 1998. Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166: 181-212.

Hargreaves, J. A. 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 34: 344-363.

Head, I. M., Hiorns, W. D., Embley, T. M., Mccarthy, A. J. and Saunders, J.R., 1993. The phylogeny of autotrophic ammonia-oxidizing bacteria as determined by analysis of 16S ribosomal RNA gene sequences. Journal of General Microbiology 139: 1147-1153.

Henriksen, K. 1980. Measurement of in situ rates of nitrification in sediment. Microbial Ecology 6: 329-337.

Heylen, K., Vanparys, B., Wittebolle, L., Verstraete, W., Boon, N. and de Vos, P. 2006. Cultivation of denitrifying bacteria: optimization of isolation conditions and diversity study. Applied and Environmental Microbiology 72(4): 2637-2643.

Horring, S. G., Montoya, J. P., Nevins, J. L., McCarthy, J. J., Ducklow, H., Goericke, R. and Malone, T. 1990. Nitrogenous nutrient transformations in the spring and fall in the Chesapeake Bay. Estuarine, Coastal and Shelf Science 30: 369-391.

Hovanec, T. A., Taylor, L. T., Blakis, A. and Delong, E. D. 1998. *Nitrospira*-Like bacteria associated with nitrite oxidation in freshwater aquaria. Applied and Environmental Microbiology 64(1): 258-264.

Hu, Q-H., Zhu, Y-M., Song, J., Li, Z-Y. and Wen, J-J. 2003. Effects of pH and Eh on release of nitrogen and phosphorus from sediments of West Lake. Journal of Zhejiang University Science 4(3): 358-362.

Hwang, B-H., Hwang, K-Y., Choi, E-S., Choi, D-K. And Jung, J-Y. 2000. Enhanced nitrite build-up in proportion to increasing alklinity/NHC_4 ratio of influent in biofilm reactor. Biotechnology Letters 22: 1287–1290.

Ignatieva, N. V. 1999. Nutrient exchange across the sediment-water interface in the eastern Gulf of Finland. Boreal Environment Research 4: 295-305.

Jackson, C. J. and Wang, Y. G. 1998. Modelling growth rate of *Penaeus monodon* Fabricius in intensively managed ponds: effects of temperature, pond age and stocking density. Aquaculture Research 29: 27–36.

Jianlong, W. and Ning, Y. 2003. Partial nitrification under limited dissolved oxygen conditions. Process Biochemistry 39(10): 1223-1229.

Jie, H., Daping, L., Yong, T., Xiaohong, H., Xiaomei, W. and Xudong, L. 2008. Effect of acetate on nitrite oxidation in mixed-population biofilms. Journal of Bioscience and Bioengineering 106(6): 580-586.

Jones, J. B., Fisher, S. G. and Grimm, N. B. 1995. Nitrification in the hyporheic zone of a desert stream ecosystem. Journal of the North American Benthological Society 14(2): 249-258.

Kaplan, D., Wilhelm, R. and Abeliovich, A. 2000. Interdependent environmental factors controlling nitrification in waters. Water Science and Technology 42(1-2): 167-172.

Kassila, J. 2003. Effects of lining and drying on the chemical composition of sediments and influence of organic carbon on crap growth in aquaculture ponds. Aquaculture Research 34: 333-343.

Kessreu, P., Kiss, I., Bihari, Z. and Polyak, B. 2003. Biological denitrification in continous-flow bioreactor containing immobilized *Pseudomonas butanovora* cells. Bioresources Technology. 87: 75-80.

Kim, D-H., Matsuda, O. and Yamamoto, T. 1997. Nitrification, denitrification and nitrate reduction rates in the sediment of Hiroshima Bay, Japan. Journal of Oceanography 53: 317-324.

Kim, D-J., Ahn, D-H. and Lee, D-I. 2005. Effects of free ammonia and dissolved oxygen on nitrification and nitrite accumulation in a biofilm airlift reactor. Korean Journal of Chemical Engineering 22(1): 85-90.

Kowalchuk, G. A., Naoumenko, Z. S., Derikx, P. J. L., Felske, A., Stephen, J. R. and Arkhipchenko, I. R. 1999. Molecular analysis of ammonia-oxidizing bacteria of the β subdivision of the class *Proteobacteria* in compost and composted materials. Applied and Environmental Microbiology 65(2): 396–403.

Krom, M. D., Porter, C. B. and Gordin, H. 1989. Nutrient budget of a marine fish pond in Eilat, Israel. Aquaculture 51: 65-80.

Kutako, M., Srisamrit, B., Arnthong, J., Powtongsook, S. and Menasveta, P. 2007. Use of nitrification biofilter on nitrogen treatment in outdoor aquaculture tank. Journal of Environmental Research 29(2): 23-46.

Lallo, R., Maharhjh, D., Gorgens, J. and Gardiner, N. 2008. Functionality of a *Bacillus cereus* biological agent in response to physiological variables encountered in aquaculture. Applied Microbiology Biotechnology 79:111–118.

Lin, Y., Tanaka, S. and Kong, H. 2006. Characterization of a newly isolated heterotrophic nitrifying bacterium. Water Practice & Technology 1(3): 112-120.

Ling, J. and Chen, S. 2005. Impact of organic on nitrification performance of different biofilters. Aquaculture Engineering 33: 150-162.

Liu, S., Yang, F., Gong, Z. and Su, Z. 2008. Assessment of the positive effect of salinity on the nitrogen removal performance and microbial composition during the start-up of CANON process. Applied and Microbiology Biotechnology 80: 339–348.

Lorenzen, K., Struve, J. and Cowan, T. J. 1997. Impact of farming intensity and water management on nitrogen dynamics in intensive pond culture: a mathematical model applied to Thai commercial shrimp ponds. Aquaculture Reseach 28: 493–508.

Mateju, V., Cizinska, S., Krejci, J. and Janoch, T. 1992. Biological water denitrification--A review. Enzyme and Microbial Technology 14: 170-183.

May, R. M. 1975. Pattern of species abundance and diversity. In Cody, M. L. and Diamond, J. M., Ecology and Evolution of Communities. Belknap Press, Cambridge, 81-120 pp.

McCaig , A.E., Phillips, C. J., Stephen, J. R. and Kowalchuk, G. A. 1999. Nitrogen cycling and community structure of proteobacterial β-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments. Applied and Environmental Microbiology 165: 213-220.

Menasveta, P., Panritdam, T., Sihanonth, P., Powtongsook, S., Chuntapa, B. and Lee, P. 2001. Design and function of a closed, recirculating seawater system with denitrification for the culture of black tiger shrimp broodstock. Aquacultural Engineering 25: 35–49.

Michauda, L., Blanchetonb, P. J., Brunia, V. and Piedrahitac, R. 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquacultural Engineering 34(3): 224-233.

Moriarty, D. J. W. 1997. The role of microorganisms in aquaculture ponds. Aquaculture 151: 333-349.

Munsiri, P., Boyd, C. E. and Hajek, B. J. 1995. Physical and chemical characteristics of bottom soil profiles in ponds at Auburn, Alabama, USA, and a proposed method for describing pond soil horizons. Journal of World Aquaculture Society 26: 346-377.

Muyzer, G., De Waal, E.C. and Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695–700.

Muyzer, G. and Smalla, K. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek 73: 127-141.

Nielsen, T. H., Nielsen, L. P. and Revsbech, N. P., 1996. Nitrification and coupled nitrification–denitrification associated with a soil-manure interface. Soil Science Society of America Journal 60, 1829–1840.

Nikolcheva, L. G., Cockshutt, A. M. and Bärlocher, F. 2003. Determining diversity of freshwater fungi on decaying leaves: comparison of traditional and molecular approaches. Applied and Environmental Microbiology 69(5): 2548–2554.

Nimrat, S. Suksawat, S., Maleeweach, P. and Vuthiphandchai, V. 2008. Effect of different shrimp pond bottom soil treatments on the change of physical characteristic and pathogenic bacteria in pond bottom soil. Aquaculture 285: 123-129.

Otte, G. and Rosenthal, H. 1979. Management of a closed brackish water system for high-density fish culture by biological and chemical water treatment. Aquaculture 18: 169-186.

Pace, N.R. 1997. A molecular view of microbial diversity and the biosphere. Science 276: 734-740.

Pauer, J. J. and Auer, M. T. 2000. Nitrification in the water column and sediment of a hypereutrophic lake and adjoining river system. Water Research 34(4): 1247-1254.

Pesaro, M., Nicollier, G., Zeyer, J. And Widmer, F. (2004). Impact of soil drying-rewetting stress on microbial communities and activities on degradation of two crop protection products. Applied and Environmental Microbiology 70(5): 2577-2587.

Porrello, S., Ferrari, G., Lenzi, M. And Persia, E. 2003. Ammonia variations in phytotreatment ponds of land-based fish farm wastewater. Aquaculture 219: 485–494

Porter, C. B., Krom, M. D., Robbins, M. G., Brickell, L. and Davidson, A. 1987. Ammonia excretion and total N budget for gilthead seabream *Sparus aurata* and its effect on water quality conditions. Aquaculture 66: 287–297.

Poxton, M. G. and Allouse, S. B., 1987. Cyclical fluctuations in ammonia and nitrite–nitrogen resulting from the feeding of turbot, *Scophthalmus maximus* (L.) in recirculating systems. Aquacultural Engineering 6: 301– 322.

Prosser, J. J. 1986. Nitrification. IOR Press. Oxford. 217 p.

Qingyun, Y., Yuhe, Y., Weisong, F., Zhigang, Y. and Hongtao, C. 2008. Plankton community composition in the Three Gorges Reservoir Region revealed by PCR-DGGE and its relationships with environmental factors. Journal of Environmental Sciences 20: 732-738.

Qiu, S. and McComb, A. J. 1996. Drying-induced stimulation of ammonium release and nitrification in reflooded lake sediment. Marine and Freshwater Research 47(3): 531 – 536.

Raven, J., Wollenweber, B. and Handley, L. 1992. A composition of ammonium and nitrate as nitrogen sources for photolithotrophs. New Phytologist 121(1): 19-32.

Rene, E. R., Kim, S. J. and Park, H. S. 2008. Effect of COD/N ratio and salinity on the performance of sequencing batch reactors. Bioresource Technology 99: 839–846.

Riise, J. C. and Roos, N. 1997. Benthic metabolism and the effects of bioturbation in a fertilized polyculture fish pond in northeast Thailand. Aquaculture 150: 45–62.

Risgaard-Petersen, N., Nicolaisen, M. H., Revsbech, N. P. and Lomstein, B. A. 2006. Competition between ammonia-oxidizing bacteria and benthic microalgae. Applied and Environmental Microbiology 70(9): 5528-5537.

Rittmann, B. E. 2006. Microbial ecology to manage processes in environmental biotechnology. Trends in Biotechnology 24(6): 261-266.

Rosselló-Mora, R., B. Thamdrup, H. Schäfer, R. Weller, and R. Amann. 1999. The response of the microbial community of marine sediments to organic carbon input under anaerobic conditions. Systematic and Applied Microbiology 22:237-248.

Ruffier, P. J., Boyle, W. C. and Wleinschmidt, J. 1981. Short-term acute bioassays to evaluate ammonia toxicity and effluent standards. Journal of the Water Pollution Control Federation 53: 367– 377.

Sakai, K., Nakamura, K., Wakayama, M. and Moriguchi, M. 1997. Change in nitrite conversion direction from oxidation to reduction in heterotrophic bacteria depending on the aeration conditions. Journal of Fermentation and Bioengineering 84(1): 47-52.

Sakami, Y., Fujioka, Y. and Shimodo, T. 2008. Comparison of microbial community structures in intensive and extensive shrimp culture ponds and a mangrove area in Thailand. Fisheries Science 74: 889-898.

Savin, M. C., Martin, J. L., LeGresley, M. Giewat, M. and Rooney-Varga, J. 2004. Plankton diversity in the Bay of Fundy as measured by morphological and molecular methods. Microbial Ecology 48: 51-65.

Sayler, G. S., Shiaris, M. P., Beck, T. W. and Held, S. 1982. Effects of polychlorinated biphenyls and environmental biotransformation products on aquatic nitrification. Applied and Environmental Microbiology 43(4): 949-952.

Schinner, F., Ohlinger, R., Kandeler, E. and Margesin, R. 1996. Methods in soil biology. Springer-Verlag Berlin Heidelberg. 426 p.

Seitzinger, S.P., Nixon, S.W. and Pilson, M.E.Q. 1984. Denitrification and nitrous oxide production in a coastal marine ecosystem. Limnology Oceanography 29: 73–83.

Semerci, N. and Cecen, F. 2007. Importance of cadmium speciation in nitrification inhibition. Journal of Hazardous Materials 147: 503-512.

Seo, J. and Boyd, C. E. 2003. Effects of bottom soil management practices on water quality improvement in channel catfish *Ictalurus punctatus* ponds. Aquaculture Engineering 25: 83-97.

Sesuk, T., Powtongsook, S. and Nootong, K. 2009. Inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters. Bioresource Technology 100: 2088–2094.

Sevrin-Reyssac, J. and Pletikosic, M. 1990. Cyanobacteria in fish ponds. Aquaculture 88: 1–20.

Shan, H. and Abbord, J. P. 2001. Ammonia removal from prawn aquaculture water using immobilized nitrifying bacteria. Applied Microbiology Biotechnology 57:791–798.

Sherry L. Dollhopf, S. L., Hyun, J-H., Smith, A. C., Adams, H. J. O'Brien, S. and Kostka. J.E. 2005. Quantification of ammonia-oxidizing bacteria and factors controlling nitrification in salt marsh sediments. Applied and Environmental Microbiology 71(1): 240–246.

Shrimali, M. and Singh, K. P. 2001. New methods of nitrate removal from water. Environmental Pollution 112: 351–359.

Sobieszuk P. and Szewczyk K. W. 2006. Estimation of (COD/NOx-N) ratio for microbial denitrification. Environmental Technology 27(1): 103-108.

Sonnenholzner, S. and Boyd, C. E. 2000. Vertical gradient of organic matter concentration and respiration rate in pond soils. Journal of the World Aquaculture Society 31: 376-380.

Strickland, J. D. and Parson, T. R. 1972. A practical handbook of seawater analysis. Fishery Research Board of Canada. Otawa. 310 p.

Suzuki, Y., Maruyamaa, T., Numataa, H., Sato, H. and Asakawa, M. 2003. Performance of a closed recirculating system with foam separation, nitrification and denitrification units for intensive culture of eel: towards zero emission. Aquacultural Engineering 29: 165–182.

Tamaki, H., Sekiguchi, Y., Hanada, S., Nakamura, K., Nomura, N., Matsumura, M. and Kamagata, Y. 2005. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. Applied and Environmental Microbiology 71(4): 2162–2169.

Tarre, S. and Green, M. 2004. High-rate nitrification at low pH in suspended and attached biomass reactors. Applied and Environmental Microbiology 70(11): 6481–6487.

Terada, A., Hibiya, K., Nagai, J., Tsuneda, S. and Hirata, A. 2003. Nitrogen removal characteristics and biofilm analysis of a membrane-aerated biofilm reactor applicable to high-strength nitrogenous wastewater treatment. Journal of Bioscience and Bioengineering 95(2): 170–178.

Thurston, R. V., Russo, R. C., Luedtke, R. J., Smith, C. E., Meyn, E. L., Chakoumakos, C., Wang, K. C. and Rown, C. J. D., 1984. Chronic toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 113: 56– 73.

Timmons, M. B., Ebeling, J. M., Weaton, F. W., Summerfelt, S. T. and Vinci, B. J. 2002. Recirculating aquaculture systems. 2nd edition, Cayuga Aqua Ventures, USA, 757.

Tomiyama, H., Ohshima, M., Ishii, S., Satoh, K., Takahashi, R. and Isobe, K. 2001. Characteristics of newly isolated nitrifying bacteria from rhizoplane of paddy rice. Microbes and Environments 16(2): 101-108.

Torsvik, V., Sorheim, R. and Goksoyr, J. 1996. Total bacterial diversity in soil and sediment communities-a review. Journal of Industrial Microbiology 17: 170-178.

Torsvik, V., Øvreås, L. and Thingstad, T. F. 2002. Prokaryotic diversity-magnitude, dynamics and controlling factors. Science. 296: 1064-1066.

Tucker, C. S. and van der Ploeg, M. 1993. Seasonal changes in water quality in commercial chanel catfish ponds in Mississippi. Journal of World Aquaculture Society 24: 473-481.

Tuominen, L., Lehtonen, K. K., Haahti, H., Hietanen, S. And Kuparinen, J. 1999. Nutrient fluxes, pore water profiles and denitrification in sediment influenced by algal sedimentation and bioturbation by *Monoporeia affinis*. Estuarine, Coastal and Shelf Science 49: 83–97.

Uygur, A. 2006. Specific nutrient removal rates in saline wastewater treatment using sequencing batch reactor. Process Biochemistry 41: 61–66.

van Benthum, W. A. J., Van Loosdrecht, M. D. M. and Heijnen, J. J. 1997. Control of heterothrophic layer formation on nitrifying biofilms in a biofilm airlift suspension reactor. Biotechnology and Bioengineering 53: 397-405.

van Gestel, M. Merckx, R. And Vlassak, K. 1992. Microbial biomass response to seasonal change and imposed drying regimes at in creasing depths of undisturbed topsoil profiles. Soil Biology & biochemistry 24: 103-111.

van Gestel, M., Merckx, R. and Vlassak, K. 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast and slow growing microorganisms in soils from different climates. Soil Biology & biochemistry 25: 109-123.

van Hannen, E. R., van Agtervld, M. P., Gons, H. J. and Laanbrok, H. J. 1998. Revealing genetic diversity of eukaryotic microorganisms in aquatic environments by denaturing gradient gel electrophoresis. Journal of Phycology 34: 206-213.

van Rijin, J., Shilo, M. and Diab, S. 1986. Phytoplankton succession in relation to the nitrogen regime in shallow, brackish-water fish ponds. Arch Hydrobiology 111: 183-186.

van Rijn, J., 1996. The potential for integrated biological treatment systems in recirculating fish culture—a review. Aquaculture 139: 181–201.

van Rijn, J., Tal, Y., Schreier, H.J., 2006. Denitrification in recirculating systems: theory and applications. Aquacultural Engineering 34: 364–376.

Verhagen, F. J. M., Duyts, H. and Laanbroek, H. J. 1992. Competition for ammonium between nitrifying and heterotrophic bacteria in continuously percolated soil columns. Applied and Environmental Microbiology 10: 3308-3311.

Visscher, P. T. and Duerr, E. O. 1991. Water quality and microbial dynamic in shrimp ponds receiving bagasse-based feed. Journal of World Aquaculture Society 22: 65-76.

Ward, D. M., Weller, R. and Bateson, M. M. 1990. 16S rDNA sequences reveal numerous uncultured microorganisms in a community. Nature 345: 63–65.

Wasielesky, W., Atwood, H., Stokes, A., and Browdy, C.L. 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258: 396–403.

Wawer, C., Jetten, M. S. M., Muyzer, G. 1997. Genetic diversity and expression of the [NiFe] hydrogenase large-subunit gene of *Desulfovibrio* spp. in environmental samples. Applied and Environmental Microbiology 63:4360-4369.

Welander, U., Henrysson, T. and Welander, T., 1998. Biological nitrogen removal from municipal landfill leachate in a pilot scale suspended carrier biofilm process. Water Research 32: 1564–1570.

Wett, B. and Rauch, W. 2003. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. Water Research 37(5): 1100-1110.

Wleringa, E. B. A., Overmann, J. and Cypionka, H. 2000. Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environmental Microbiology 2(4): 417-427.

Wu, J. and Brookes, P. C. 2005. The proportional mineralization of microbial biomass and organic matter caused by air-drying and rewetting of a grassland soil. Soil Biology & Biochemistry 37: 507-515.

Xia, L. Z., Yang, L.Z. and Yan, M.C. 2004. Nitrogen and phosphorus cycling in shrimp ponds and the measures for sustainable management. Environmental Geochemistry and Health 26: 245–251.

Xia, S., Shi, Y., Fu, Y, and Ma, X. 2005. DGGE analysis of 16S rDNA of ammonia-oxidizing bacteria in chemical-biological flocculation and chemical coagulation systems. Applied Microbiology and Biotechnology 69: 99-105.

Zhang, L-H., Jia, J-P., Ying, D-W, Zhu, N-W., Zhu, Y-C., Zhu. S. and Chen, S. 2002. Electrochemical effect on denitrification in different microenvironments around anodes and cathodes. Research in Microbiology 156: 88-92.

Zhang, Y., Zhou, J., Guo, J., Zhang, X., Zhao, L. and Yuan, S. 2008. Study on nitrite accumulation characteristics and nitrifying population dynamics at different growth environments. Proceeding of World Academy of Science, Engineering and Technology 34: 883-887.

Zhu, S. and Chen, S. 1999. An experimental study on nitrification biofilm performance using a series reactor system, Aquacultural Engineering 20: 245–259.

Zhu, S. and Chen, S. 2002. The impact of temperature on nitrification rate in fixed film biofilters. Aquacultural Engineering 26: 221-237.

APPENDICES

APPENDIX A

STANDARD CURVE OF AMMONIA, NITRITE, NITRAE AND PHOSPHATE ANALYSIS

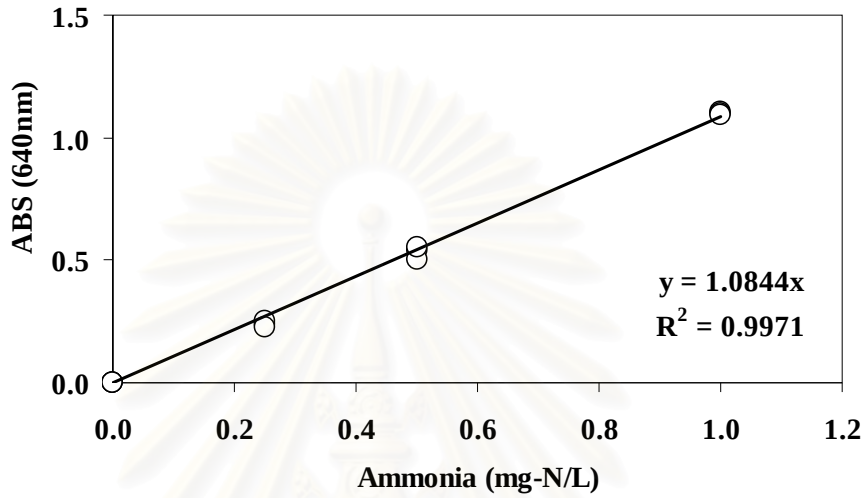


Figure A-1 Standard curve of ammonia (NH_4^+-N).

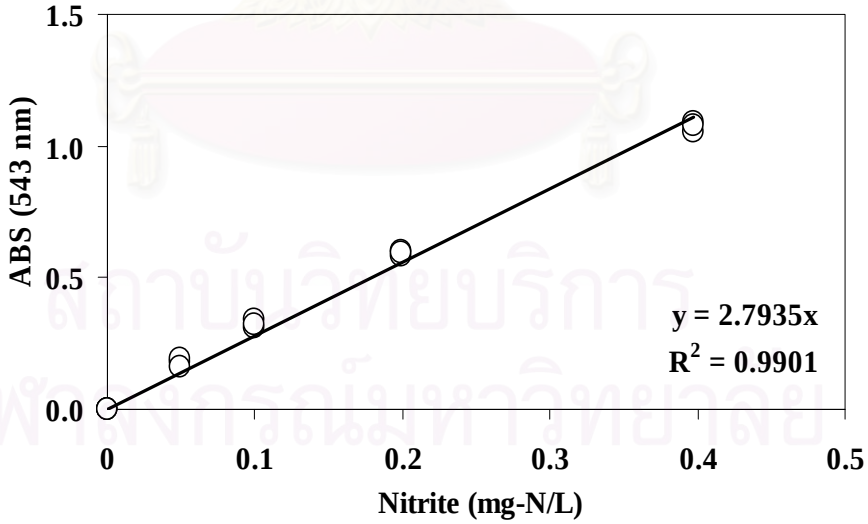


Figure A-2 Standard curve of nitrite (NO_2^--N).

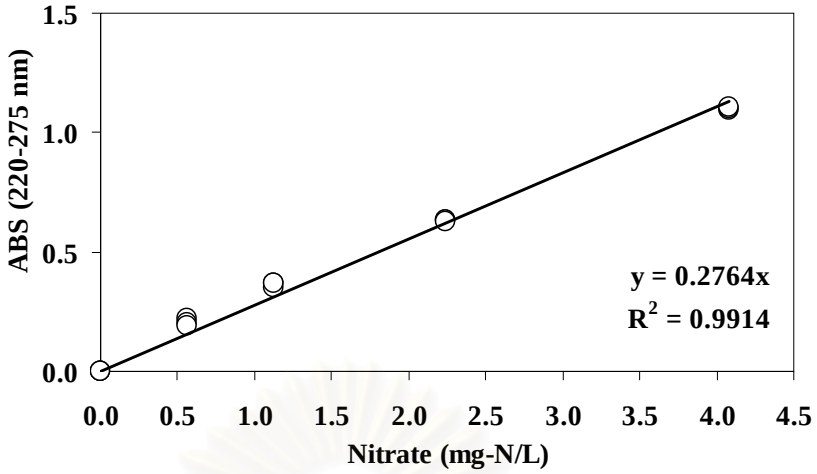


Figure A-3 Standard curve of nitrate (NO_3^--N).

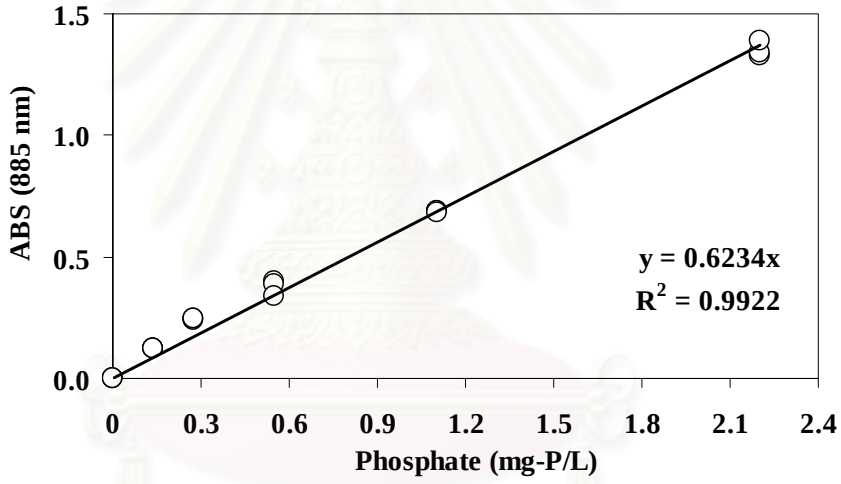


Figure A-4 Standard curve of phosphate (PO_4^{2-}-P).

APPENDIX B

CONCENTRATION OF INORGANIC NITROGEN AND PHOSPATE IN SEDIMENT FROM SHRIMP POND UNDER LABORATORY CONDITION

Table B-1 Concentration of ammonia, nitrite, nitrate and phosphate in water column from the low salinity (5 PSU) chamber.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)		Phosphate (mg-P/L)	
	Average	SD	Average	SD	Average	SD	Average	SD
0	0.20	0.01	0.27	0.06	0.07	0.06	0.32	0.02
1	0.30	0.00	0.11	0.01	0.13	0.01	0.49	0.00
2	0.43	0.03	0.10	0.04	0.24	0.04	0.71	0.04
3	0.44	0.03	0.08	0.04	0.19	0.04	0.73	0.06
4	0.37	0.03	0.07	0.04	0.37	0.04	0.05	0.02
5	0.58	0.11	0.05	0.01	0.43	0.01	0.04	0.00
6	0.39	0.03	0.27	0.02	0.38	0.02	0.01	0.00
7	0.75	0.07	0.38	0.02	1.27	0.02	0.01	0.00
9	0.51	0.09	0.43	0.00	1.31	0.00	0.07	0.00
10	0.36	0.06	0.38	0.02	1.18	0.02	0.03	0.00
11	0.04	0.00	0.21	0.02	1.32	0.02	0.06	0.01
12	0.03	0.01	0.09	0.01	1.13	0.00	0.06	0.00
13	0.01	0.00	0.04	0.01	1.16	0.00	0.02	0.00
14	0.11	0.06	0.02	0.00	1.08	0.00	0.02	0.01
15	0.12	0.04	0.02	0.00	1.17	0.01	0.01	0.01
16	0.09	0.03	0.11	0.01	0.96	0.00	0.06	0.07
22	0.04	0.00	0.01	0.00	1.20	0.01	0.07	0.04
25	0.08	0.01	0.02	0.00	1.50	0.00	0.04	0.02
28	0.04	0.01	0.01	0.00	1.46	0.00	0.04	0.01
31	0.05	0.00	0.01	0.00	1.65	0.00	0.32	0.02

Table B-2 Concentration of ammonia, nitrite, nitrate and phosphate in water column from the high salinity (20 PSU) chamber.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)		Phosphate (mg-P/L)	
	Average	SD	Average	SD	Average	SD	Average	SD
0	0.14	0.01	0.21	0.06	0.60	0.01	0.30	0.02
1	0.36	0.04	0.11	0.01	0.94	0.00	0.49	0.00
2	0.53	0.01	0.08	0.04	1.11	0.03	0.73	0.04
3	0.65	0.06	0.07	0.04	1.05	0.03	0.76	0.06
4	0.44	0.02	0.06	0.04	1.12	0.03	0.04	0.02
5	0.49	0.12	0.06	0.01	0.80	0.11	0.04	0.00
6	0.80	0.01	0.29	0.02	0.77	0.03	0.01	0.00
7	0.29	0.01	0.51	0.02	1.87	0.07	0.01	0.00
9	0.24	0.03	0.70	0.00	1.80	0.09	0.06	0.00
10	0.05	0.01	0.60	0.02	2.36	0.06	0.02	0.00
11	0.05	0.01	0.12	0.02	2.32	0.00	0.04	0.01
12	0.01	0.00	0.01	0.00	1.99	0.01	0.06	0.00
13	0.01	0.00	0.00	0.00	2.04	0.00	0.02	0.00
14	0.09	0.02	0.01	0.00	2.06	0.06	0.03	0.01
15	0.08	0.01	0.02	0.01	2.07	0.04	0.01	0.01
16	0.04	0.00	0.01	0.00	1.59	0.03	0.01	0.07
22	0.01	0.01	0.01	0.01	1.99	0.00	0.03	0.00
25	0.06	0.01	0.02	0.00	2.15	0.01	0.03	0.00
28	0.02	0.00	0.01	0.00	2.08	0.01	0.04	0.01
31	0.02	0.01	0.01	0.00	2.05	0.00	0.04	0.01

Table B-3 Concentration of ammonia, nitrite and nitrate in pore water from the low salinity chamber.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	25.00	0.94	4.64	0.56	0.01	0.00
4	4.06	0.10	0.92	0.19	0.00	0.00
7	1.50	0.06	0.40	0.03	0.00	0.00
10	2.48	0.27	0.50	0.02	0.01	0.00
13	18.40	0.30	3.81	0.10	0.07	0.00
15	17.09	0.57	1.27	0.37	0.03	0.00
22	16.22	0.14	4.45	0.22	0.05	0.00
25	16.78	0.19	2.29	0.23	0.04	0.00
28	18.74	0.66	1.57	0.07	0.01	0.00
31	21.26	0.49	0.75	0.03	0.02	0.00

Table B-4 Concentration of ammonia, nitrite and nitrate in pore water from the high salinity chamber.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	16.00	0.94	4.64	0.56	0.01	0.00
4	4.06	0.10	0.92	0.19	0.00	0.00
7	1.50	0.06	0.40	0.03	0.00	0.00
10	2.48	0.27	0.50	0.02	0.01	0.00
13	28.40	0.30	3.81	0.10	0.07	0.00
15	7.09	0.57	1.27	0.37	0.03	0.00
22	16.22	0.14	4.45	0.22	0.05	0.00
25	16.78	0.19	2.29	0.23	0.04	0.00
28	18.74	0.66	1.57	0.07	0.01	0.00
31	21.26	0.49	0.75	0.03	0.02	0.00

Table B-5 Concentration of ammonia, nitrite and nitrate in extracted sediment from the low salinity chamber.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	25.58	2.57	2.09	0.50	5.58	0.60
4	19.25	1.52	5.44	1.19	16.47	2.00
7	6.22	0.83	1.72	0.04	10.35	0.15
10	10.40	0.25	1.32	0.08	55.37	5.00
13	31.67	0.32	1.64	0.44	53.68	0.43
15	40.55	3.38	2.20	0.25	32.19	0.26
22	52.69	1.30	0.96	0.18	40.92	8.90
25	13.18	0.15	0.75	0.08	22.48	3.50
28	20.43	1.04	1.26	0.05	36.58	3.00
31	22.15	0.63	0.66	0.03	27.25	0.45

Table B-6 Concentration of ammonia, nitrite and nitrate in extracted sediment from the high salinity chamber.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	25.58	0.13	2.09	0.50	5.577652	2.00
4	10.40	0.06	6.61	1.45	13.3312	4.30
7	4.67	0.01	1.79	0.05	8.724186	3.00
10	13.55	0.02	2.25	0.06	27.17432	7.80
13	75.24	0.07	2.34	0.12	18.29041	0.99
15	59.54	0.11	2.45	0.10	33.49891	6.50
22	48.85	0.05	0.93	0.01	37.32736	3.00
25	11.46	0.01	0.31	0.02	9.117406	0.40
28	8.41	0.05	0.33	0.02	19.76653	0.30
31	3.12	0.10	1.23	0.12	22.05708	2.30

Table B-7 Concentration of ammonia, nitrite and nitrate in water column from control chamber supplied with low dissolved oxygen (approximately 2.5 mg-O_2/L).

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	3.52	0.35	0.07	0.02	1.44	0.05
1	3.95	1.06	0.07	0.02	1.23	0.09
2	3.69	0.75	0.07	0.02	1.17	0.21
3	3.72	0.79	0.16	0.07	1.55	0.08
5	4.39	0.86	0.93	0.38	1.83	0.21
6	2.29	0.98	1.54	0.37	3.18	0.54
7	1.04	0.80	3.27	0.18	4.12	0.24
8	0.02	0.02	3.41	0.33	4.21	0.26
10	0.05	0.05	2.63	0.13	3.75	0.09
12	0.00	0.00	2.58	0.15	3.18	0.30
14	0.00	0.00	1.65	0.14	3.05	0.17
16	0.00	0.00	1.66	0.12	2.69	0.16
18	0.00	0.00	0.43	0.15	2.69	0.21
20	0.00	0.00	0.01	0.00	2.91	0.56
22	0.00	0.00	0.01	0.00	2.60	0.11
24	0.00	0.01	0.02	0.03	3.07	0.89
26	0.04	0.01	0.01	0.01	2.27	0.05
28	0.06	0.01	0.02	0.02	2.15	0.07
29	0.11	0.04	0.03	0.01	1.61	0.36
30	0.49	0.06	0.12	0.05	1.67	0.17
31	0.74	0.08	0.07	0.04	1.65	0.29
32	0.87	0.08	0.04	0.04	1.24	0.03
33	0.41	0.15	0.01	0.01	1.03	0.13
34	0.21	0.12	0.00	0.00	1.11	0.03
35	0.09	0.06	0.00	0.00	1.16	0.06

Table B-8 Concentration of ammonia, nitrite and nitrate in water column from treatment chamber supplied with high dissolved oxygen to 7 mg-O_2/L.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	2.44	0.39	0.27	0.02	1.62	0.01
1	3.02	0.94	0.26	0.02	1.40	0.02
2	2.33	0.29	0.53	0.07	1.80	0.17
3	1.36	0.33	0.87	0.04	2.36	0.23
5	0.38	0.16	1.69	0.06	3.35	0.24
6	0.01	0.01	1.55	0.22	3.03	0.49
7	0.00	0.00	3.32	0.25	3.13	0.34
8	0.00	0.01	3.94	0.32	3.14	0.19
10	0.00	0.00	2.68	0.32	3.01	0.34
12	0.01	0.01	2.59	0.20	2.86	0.55
14	0.04	0.03	1.68	0.10	2.80	0.40
16	0.01	0.02	1.27	0.33	2.92	0.36
18	0.01	0.02	0.37	0.22	3.14	0.18
20	0.01	0.02	0.08	0.07	3.26	0.12
22	0.01	0.02	0.09	0.10	3.37	0.24
24	0.00	0.00	0.01	0.00	3.50	0.14
26	0.04	0.02	0.02	0.02	3.03	0.21
28	0.00	0.00	0.02	0.01	2.81	0.20
29	0.05	0.03	0.01	0.01	2.46	0.11
30	0.32	0.06	0.02	0.03	2.09	0.07
31	0.42	0.08	0.04	0.03	1.77	0.03
32	0.58	0.07	0.01	0.01	1.39	0.05
33	0.07	0.02	0.01	0.01	1.24	0.01
34	0.19	0.16	0.01	0.01	1.30	0.02
35	0.02	0.01	0.01	0.01	1.24	0.04

Table B-9 Concentration of ammonia, nitrite and nitrate in water from the control chamber that had no $NaHCO_3$ addition.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.86	0.31	0.03	0.00	0.12	0.10
1	1.31	0.06	0.06	0.02	Nd*	Nd*
2	1.57	0.49	0.04	0.01	Nd*	Nd*
3	1.75	0.18	0.07	0.01	Nd*	Nd*
4	1.70	0.23	0.13	0.02	Nd*	Nd*
5	1.97	0.09	0.27	0.07	Nd*	Nd*
6	1.43	0.21	0.52	0.20	Nd*	Nd*
7	0.85	0.47	0.97	0.27	1.40	1.12
8	1.52	0.23	1.98	0.10	Nd*	Nd*
9	1.16	0.17	1.89	0.13	Nd*	Nd*
10	0.94	0.12	1.75	0.20	Nd*	Nd*
11	0.51	0.12	1.32	0.33	Nd*	Nd*
12	0.21	0.11	1.33	0.18	Nd*	Nd*
13	0.48	0.10	1.30	0.17	Nd*	Nd*
14	0.51	0.20	1.22	0.24	0.41	0.15
15	0.34	0.16	1.19	0.31	Nd*	Nd*
16	0.19	0.03	1.03	0.28	6.20	0.97
18	0.07	0.02	0.62	0.34	4.72	1.51
19	0.11	0.08	0.43	0.32	3.38	1.24
21	0.03	0.01	0.02	0.01	2.31	0.78
29	0.09	0.04	0.03	0.03	0.46	0.27

Remark: Nd* was not detected.

Table B-10 Concentration of ammonia, nitrite and nitrate in water from the treatment chamber, here it was added with 0.105 g $NaHCO_3$/L that provided the C:N ratio of 2:1.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	1.09	0.18	0.04	0.01	0.09	0.04
1	1.12	0.12	0.05	0.00	Nd*	Nd*
2	1.12	0.25	0.05	0.02	Nd*	Nd*
3	1.44	0.29	0.08	0.04	Nd*	Nd*
4	1.18	0.13	0.12	0.07	Nd*	Nd*
5	1.34	0.18	0.29	0.16	Nd*	Nd*
6	1.03	0.50	0.47	0.13	Nd*	Nd*
7	1.19	0.28	1.09	0.33	0.91	0.39
8	1.28	0.48	1.71	0.46	Nd*	Nd*
9	1.10	0.09	1.50	0.21	Nd*	Nd*
10	1.00	0.22	1.43	0.09	Nd*	Nd*
11	0.47	0.12	0.94	0.05	Nd*	Nd*
12	0.09	0.07	0.96	0.16	Nd*	Nd*
13	0.26	0.04	0.91	0.08	Nd*	Nd*
14	0.34	0.00	1.02	0.15	1.62	0.48
15	0.18	0.03	0.96	0.17	Nd*	Nd*
16	0.21	0.03	0.68	0.19	4.06	1.11
18	0.06	0.02	0.47	0.17	3.85	0.86
19	0.06	0.02	0.33	0.15	2.98	0.57
21	0.03	0.01	0.02	0.01	1.06	1.46
29	0.14	0.06	0.05	0.05	0.49	0.16

Remark: Nd* was not detected.

Table B-11 Concentration of ammonia, nitrite and nitrate in pore water from the control chamber that was not applied with $NaHCO_3$.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	1.26	0.00	0.23	0.00	1.44	0.00
6	140.77	42.42	5.01	4.68	Nd*	Nd*
9	143.40	59.97	0.36	0.17	8.33	1.63
15	3.32	0.50	0.14	0.02	11.39	7.01
18	4.47	0.31	0.19	0.05	Nd*	Nd*
21	3.75	0.87	0.33	0.04	5.28	1.00
29	3.50	1.05	0.15	0.06	0.01	0.02

Remark: Nd* was not detected.

Table B-12 Concentration of ammonia, nitrite and nitrate in pore water from the treatment chamber that was applied with 0.105 g $NaHCO_3$/L that provided the C:N ratio of 2:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	1.26	0.00	0.23	0.00	1.44	0.00
6	106.69	11.40	8.62	3.28	Nd	Nd
9	147.85	34.39	0.42	0.17	20.17	3.31
15	2.78	1.38	0.10	0.04	3.98	3.45
18	3.46	2.54	0.16	0.13	Nd	Nd
21	5.01	3.98	0.66	0.63	9.29	6.64
29	2.48	1.07	0.14	0.03	0.06	0.06

Remark: Nd* was not detected.

Table B-13 Concentration of ammonia, nitrite and nitrate in extracted soil from the control chamber that was not applied with $NaHCO_3$.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	1.41	0.00	0.09	0.00	0.92	0.00
6	194.75	31.50	5.64	1.01	Nd*	Nd*
9	210.00	41.71	Nd*	Nd*	18.76	2.11
15	6.51	1.62	0.07	0.04	5.94	3.23
18	4.53	0.55	0.03	0.02	Nd*	Nd*
21	3.91	0.82	0.03	0.02	3.74	1.74
29	3.44	0.61	0.03	0.00	0.03	0.00

Remark: Nd* was not detected.

Table B-14 Concentration of ammonia, nitrite and nitrate in extracted soil from the treatment chamber that was applied with 0.105 g $NaHCO_3$/L that provided the C:N ratio of 2:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	1.41	0.00	0.09	0.00	0.92	0.00
6	154.76	19.15	11.71	1.07	Nd*	Nd*
9	245.33	63.33	Nd*	Nd*	20.11	2.38
15	5.97	2.25	0.08	0.04	5.93	1.84
18	4.79	1.03	0.05	0.01	Nd*	Nd*
21	6.15	2.26	0.04	0.02	3.73	0.78
29	4.39	1.52	0.03	0.00	0.03	0.01

Remark: Nd* was not detected.

Table B-15 Concentration of ammonia, nitrite and nitrate in water from the control chamber, here it was no added with methanol solution.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	1.55	0.56	0.23	0.01	0.97	0.18
1	1.73	0.40	0.35	0.03	0.36	0.43
2	2.50	0.07	0.30	0.10	Nd*	Nd*
3	4.05	0.93	0.03	0.02	Nd*	Nd*
4	7.65	1.37	0.16	0.00	Nd*	Nd*
6	8.07	1.97	0.08	0.00	2.78	1.61
7	6.80	2.44	0.06	0.00	Nd*	Nd*
9	8.74	2.17	0.11	0.01	1.67	0.16
12	6.32	1.93	2.17	0.13	Nd*	Nd*
13	4.66	3.71	2.79	0.27	Nd*	Nd*
14	3.76	2.70	3.69	0.43	7.60	1.45
18	0.29	0.05	5.51	1.51	10.03	2.15
21	0.82	0.48	3.52	2.70	8.10	3.08
23	0.19	0.12	4.04	1.83	10.77	0.76
25	0.16	0.05	2.96	0.12	7.93	1.54
27	0.14	0.03	2.10	0.44	6.97	0.24
29	0.18	0.06	1.49	0.59	4.38	0.50
31	0.17	0.08	0.97	2.78	3.99	0.27
33	0.31	0.09	0.30	0.57	3.93	0.53
35	0.42	0.21	0.18	0.06	2.11	0.36
39	0.06	0.01	0.13	0.53	3.73	0.30
41	0.09	0.01	0.07	0.29	4.30	0.65
43	0.10	0.01	0.04	0.17	4.15	0.13
46	0.68	0.13	0.07	0.08	4.45	0.62
48	0.47	0.11	0.05	0.09	4.37	0.56

Remark: Nd* was not detected.

Table B-16 Concentration of ammonia, nitrite and nitrate in water from the treatment-1, here it was applied with methanol solution at the C:N ratio of 2:1.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	1.19	0.10	0.29	0.01	1.83	1.37
1	0.37	0.20	0.71	0.03	Nd*	Nd*
2	1.93	0.43	0.43	0.10	Nd*	Nd*
3	3.44	0.20	0.03	0.02	3.16	0.63
4	7.60	0.55	0.11	0.00	Nd*	Nd*
6	7.35	0.58	0.02	0.00	1.32	1.06
7	7.35	1.95	0.01	0.00	Nd*	Nd*
9	9.74	0.73	0.03	0.01	3.01	2.48
12	7.76	1.08	0.33	0.13	Nd*	Nd*
13	8.05	0.77	0.67	0.27	Nd*	Nd*
14	8.66	0.62	0.83	0.43	3.61	0.74
18	5.16	0.59	5.37	1.51	10.36	1.78
21	3.56	0.97	4.72	2.70	8.73	1.39
23	3.39	2.41	5.78	1.83	11.82	2.36
25	0.82	0.69	6.36	0.12	13.66	0.48
27	0.54	0.11	5.01	0.44	9.97	0.39
29	0.26	0.08	4.49	0.59	7.74	0.84
31	0.26	0.02	5.16	2.78	7.52	0.50
33	0.50	0.10	1.58	0.57	5.51	0.21
35	0.94	0.05	0.65	0.06	6.71	0.19
39	0.11	0.07	0.48	0.53	4.88	2.06
41	0.10	0.04	0.25	0.29	4.84	0.48
43	0.14	0.07	0.16	0.17	5.46	0.10
46	0.93	0.16	0.14	0.08	5.66	0.33
48	0.66	0.05	0.12	0.09	3.68	0.57

Remark: Nd* was not detected.

Table B-17 Concentration of ammonia, nitrite and nitrate in water from the treatment-2, here it was applied with methanol solution at the C:N ratio of 4:1.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.94	0.46	0.26	0.01	2.40	0.17
1	0.36	0.47	0.48	0.06	Nd*	Nd*
2	0.51	0.13	0.16	0.04	Nd*	Nd*
3	2.37	0.41	0.02	0.01	2.02	1.47
4	7.05	0.43	0.02	0.02	Nd*	Nd*
6	5.96	0.63	0.01	0.00	1.00	0.68
7	5.98	0.56	0.01	0.00	Nd*	Nd*
9	8.50	0.42	0.01	0.00	1.43	0.49
12	8.41	1.82	0.13	0.08	Nd*	Nd*
13	9.90	3.20	0.20	0.13	Nd*	Nd*
14	9.33	1.37	0.17	0.02	3.09	0.82
18	9.50	2.12	1.72	1.30	4.90	1.50
21	4.01	2.05	5.31	1.12	8.95	1.56
23	2.21	1.39	7.69	1.84	14.63	2.76
25	0.75	0.32	7.66	3.01	15.01	4.22
27	0.43	0.06	5.50	2.11	10.96	4.34
29	0.27	0.13	5.03	1.67	8.24	2.17
31	0.21	0.02	3.98	1.11	8.54	0.19
33	0.38	0.26	2.45	0.47	5.54	1.00
35	0.82	0.49	1.54	0.23	6.85	0.62
39	0.30	0.42	0.87	0.90	3.50	0.38
41	0.23	0.08	0.59	0.66	4.76	1.23
43	0.23	0.12	0.31	0.30	5.04	0.96
46	0.64	0.23	0.13	0.07	5.59	0.45
48	0.44	0.10	0.09	0.03	5.12	0.73

Remark: Nd* was not detected.

Table B-18 Concentration of ammonia, nitrite and nitrate in pore water from the control chamber; here it was no added with methanol solution.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	72.84	21.30	2.77	4.44	12.83	0.07
6	117.13	92.29	1.85	1.54	11.13	0.05
25	32.84	33.79	3.27	2.95	9.57	0.02
48	14.80	8.12	2.13	2.35	18.73	0.02

Table B-19 Concentration of ammonia, nitrite and nitrate in pore water from the treatment-1 that was added with methanol solution at the C:N ratio of 2:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	116.09	144.18	0.09	0.05	17.07	0.06
6	56.24	14.65	0.22	0.03	7.65	0.04
25	9.09	4.97	0.43	0.13	8.70	0.01
48	12.91	2.19	0.35	0.17	7.82	0.01

Table B-20 Concentration of ammonia, nitrite and nitrate in pore water from the treatment-2 that was added with methanol solution at the C:N ratio of 4:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	106.64	22.30	0.08	0.07	21.76	0.09
6	39.54	10.08	0.18	0.02	12.03	0.09
25	6.88	4.00	0.34	0.04	4.99	0.03
48	15.53	5.72	0.38	0.02	8.40	0.00

Table B-21 Concentration of ammonia, nitrite and nitrate in extracted soil from the control chamber; here it was no added with methanol solution.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	45.35	3.58	6.53	5.25	67.37	0.07
6	42.98	3.54	2.26	1.70	54.70	0.05
25	16.14	1.81	5.88	2.24	16.76	0.02
48	12.19	0.66	4.16	2.73	21.54	0.02

Table B-22 Concentration of ammonia, nitrite and nitrate in extracted soil from the treatment-1 that was added with methanol solution at the C:N ratio of 2:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	7.19	2.28	0.05	0.01	56.57	0.06
6	8.15	0.45	0.03	0.00	37.20	0.04
25	2.33	1.01	2.02	0.64	7.27	0.01
48	8.55	0.86	0.81	0.25	8.38	0.01

Table B-23 Concentration of ammonia, nitrite and nitrate in extracted soil from the treatment-2 that was added with methanol solution at the C:N ratio of 4:1.

Day	Ammonia (μg-N/g wet sediment)		Nitrite (μg-N/g wet sediment)		Nitrate (μg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	8.14	2.67	0.11	0.01	89.76	0.09
6	8.63	1.07	0.04	0.01	89.04	0.09
25	4.08	1.78	5.86	2.70	28.81	0.03
48	7.67	2.36	2.27	0.71	4.20	0.00

Table B-24 Concentration of ammonia (mg-N/L), nitrite (mg-N/L), nitrate (mg-N/L) and phosphate (mg-P/L) in the dark chamber.

Day	Ammonia		Nitrite		Nitrate		Phosphate	
	Average	SD	Average	SD	Average	SD	Average	SD
0	0.02	0.01	0.00	0.00	1.45	0.06	0.01	0.00
1	0.25	0.01	0.04	0.00	3.83	0.09	0.24	0.09
2	0.23	0.02	0.07	0.00	6.85	0.11	0.18	0.02
3	0.59	0.25	0.20	0.00	4.76	0.14	0.22	0.01
4	0.23	0.05	0.48	0.04	4.20	0.13	0.30	0.03
5	0.13	0.02	0.81	0.05	4.19	0.05	0.18	0.01
6	0.04	0.03	0.97	0.05	3.99	0.10	0.19	0.02
7	0.03	0.03	1.07	0.06	3.71	0.03	0.17	0.03
8	0.00	0.00	1.17	0.06	3.63	0.12	0.15	0.01
9	0.00	0.00	0.96	0.07	3.48	0.03	0.18	0.03
10	0.02	0.01	0.87	0.07	3.86	0.10	0.12	0.02
11	0.03	0.01	0.55	0.01	3.68	0.01	0.12	0.01
12	0.04	0.01	0.38	0.01	3.99	0.04	0.17	0.01
13	0.01	0.02	0.21	0.02	3.96	0.08	0.09	0.03
14	0.00	0.00	0.06	0.00	4.06	0.07	0.13	0.01
15	0.00	0.00	0.01	0.00	3.87	0.03	0.56	0.02
16	0.02	0.01	0.02	0.00	3.94	0.09	0.13	0.06
17	0.07	0.00	0.19	0.01	3.28	0.01	0.43	0.01
18	0.16	0.03	0.18	0.01	3.13	0.09	0.65	0.01
19	0.12	0.04	0.21	0.00	3.39	0.02	0.20	0.03
20	0.01	0.01	0.08	0.00	3.68	0.03	0.28	0.03
21	0.02	0.02	0.01	0.00	3.66	0.05	0.27	0.02
22	0.01	0.01	0.01	0.00	3.65	0.07	0.64	0.07
23	0.00	0.00	0.01	0.00	3.23	0.03	0.30	0.05
24	0.00	0.00	0.00	0.00	3.24	0.02	0.58	0.03
25	0.01	0.01	0.00	0.00	3.17	0.03	0.21	0.01
26	0.00	0.00	0.01	0.00	3.24	0.04	0.58	0.02
27	0.00	0.00	0.01	0.00	3.17	0.03	0.20	0.02
28	0.01	0.01	0.04	0.00	2.37	0.02	0.09	0.01
29	0.20	0.03	0.04	0.00	1.30	0.00	0.05	0.01
30	0.31	0.02	0.14	0.03	1.92	0.04	0.14	0.01
31	0.40	0.08	0.27	0.01	2.14	0.03	0.12	0.02
32	0.00	0.00	0.08	0.00	2.97	0.02	0.12	0.02
33	0.36	0.18	0.07	0.00	2.34	0.04	0.18	0.03
34	0.98	0.08	0.12	0.00	2.58	0.79	0.39	0.02
35	1.68	0.28	0.37	0.05	2.31	0.04	0.64	0.02
36	0.90	0.04	0.90	0.13	2.73	0.07	0.68	0.03
37	0.25	0.02	2.04	0.12	3.01	0.11	0.52	0.01
38	0.04	0.02	1.82	0.06	3.06	0.33	0.44	0.02
39	0.10	0.02	1.18	0.05	3.48	0.05	0.39	0.04
40	0.03	0.00	0.54	0.02	3.99	0.03	0.38	0.01
41	0.02	0.01	0.06	0.01	4.11	0.08	0.41	0.01
42	0.13	0.02	0.15	0.02	4.12	0.11	0.45	0.03
43	0.37	0.00	0.21	0.02	6.74	0.10	0.29	0.00
44	0.09	0.03	0.06	0.00	5.88	0.14	0.32	0.05
45	0.15	0.01	0.06	0.01	6.26	0.07	0.29	0.01
46	0.12	0.01	0.03	0.00	4.23	0.25	0.34	0.03

Table B-25 Concentration of ammonia (mg-N/L), nitrite (mg-N/L), nitrate (mg-N/L) and phosphate (mg-P/L) in the light chamber.

Day	Ammonia		Nitrite		Nitrate		Phosphate	
	Average	SD	Average	SD	Average	SD	Average	SD
0	0.02	0.01	0.00	0.00	1.45	0.06	0.01	0.00
1	0.24	0.05	0.04	0.00	7.25	0.29	0.18	0.01
2	0.30	0.05	0.07	0.00	7.52	0.55	0.19	0.01
3	0.32	0.04	0.17	0.00	4.76	0.17	0.26	0.11
4	0.16	0.05	0.37	0.02	4.48	0.04	0.14	0.02
5	0.06	0.01	0.48	0.00	4.04	0.06	0.11	0.01
6	0.00	0.00	0.60	0.02	3.62	0.08	0.10	0.02
7	0.00	0.00	0.45	0.01	3.43	0.05	0.04	0.01
8	0.00	0.00	0.38	0.03	3.35	0.18	0.06	0.02
9	0.00	0.00	0.30	0.06	3.05	0.02	0.09	0.00
10	0.02	0.00	0.23	0.01	3.19	0.08	0.08	0.02
11	0.00	0.00	0.15	0.01	3.23	0.05	0.09	0.01
12	0.00	0.00	0.07	0.01	3.12	0.19	0.07	0.01
13	0.00	0.00	0.04	0.00	3.00	0.02	0.02	0.01
14	0.00	0.00	0.01	0.00	2.46	0.05	0.01	0.00
15	0.00	0.00	0.01	0.00	2.15	0.01	0.33	0.01
16	0.00	0.00	0.01	0.00	2.10	0.06	0.04	0.01
17	0.01	0.02	0.03	0.00	1.92	0.01	0.14	0.07
18	0.03	0.02	0.02	0.00	1.68	0.07	0.04	0.00
19	0.07	0.00	0.02	0.00	1.56	0.03	0.27	0.01
20	0.00	0.00	0.01	0.00	1.59	0.02	1.26	0.00
21	0.00	0.00	0.00	0.00	1.40	0.03	0.07	0.01
22	0.00	0.00	0.00	0.00	1.42	0.01	0.08	0.01
23	0.00	0.00	0.00	0.00	1.16	0.02	0.02	0.01
24	0.00	0.00	0.00	0.00	1.02	0.02	0.02	0.01
25	0.01	0.01	0.00	0.00	1.06	0.05	0.30	0.08
26	0.00	0.00	0.00	0.00	1.33	0.02	0.27	0.08
27	0.00	0.00	0.00	0.00	1.25	0.03	0.04	0.01
28	0.00	0.00	0.00	0.00	1.05	0.02	0.02	0.01
29	0.13	0.01	0.00	0.00	1.17	0.03	0.00	0.00
30	0.14	0.04	0.01	0.01	1.09	0.02	0.00	0.00
31	0.20	0.01	0.01	0.00	1.26	0.01	0.00	0.00
32	0.23	0.02	0.04	0.00	1.27	0.02	0.02	0.00
33	0.33	0.05	0.02	0.00	1.72	0.05	0.11	0.00
34	1.23	0.13	0.02	0.00	1.54	0.02	0.32	0.01
35	1.82	0.26	0.04	0.00	1.71	0.04	0.56	0.03
36	1.76	0.15	0.05	0.00	1.74	0.02	0.65	0.01
37	3.14	0.52	0.09	0.01	1.89	0.07	0.52	0.05
38	3.15	0.94	0.14	0.01	2.05	0.04	0.55	0.02
39	2.35	0.23	0.20	0.01	2.06	0.05	0.50	0.00
40	1.99	0.09	0.41	0.00	2.29	0.02	0.42	0.03
41	2.00	0.09	1.28	0.08	2.26	0.10	0.35	0.01
42	0.86	0.05	3.18	0.15	3.25	0.07	0.22	0.02
43	0.02	0.01	4.02	0.06	4.95	0.14	0.10	0.01
44	0.02	0.01	2.87	0.09	4.15	0.11	0.09	0.01
45	0.03	0.01	1.71	0.06	2.80	0.14	0.09	0.01
46	0.00	0.00	0.90	0.26	2.99	0.08	0.03	0.01

Table B-25 Concentration of chlorophyll-*a* in dark and in light chamber during 46 days of experiment.

Day	Chlorophyll-*a* (mg-N/L)	
	Light chamber	**Dark chamber**
0	74.88	74.88
1	74.88	24.04
8	73.45	0.00
16	39.58	1.97
17	11.60	10.12
18	4.64	2.26
19	12.50	0.00
20	32.32	0.00
21	78.38	0.00
22	29.55	0.00
23	2.32	8.91
24	11.05	0.00
25	0.00	2.69
27	20.24	41.69
28	80.12	45.35
29	67.89	14.47
32	95.26	66.96
33	100.08	27.63
38	144.43	12.82
39	353.93	19.17
40	115.58	16.53
41	64.67	41.73
43	223.67	46.73
44	734.27	58.23
45	190.27	29.06
46	232.00	28.06

APPENDIX C

CONCENTRATION OF INORGANIC NITROGEN IN SEDIMENT FROM SHRIMP POND THAT TREATED BY DRYING METHOD

Table C-1 Concentration of ammonia, nitrite and nitrate in water column from the control chamber packed with untreated wet sediment.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.46	0.13	0.09	0.02	1.16	0.26
1	0.47	0.21	0.16	0.02	1.29	0.07
2	0.60	0.27	0.21	0.03	1.27	0.09
3	0.64	0.27	0.29	0.06	1.27	0.13
4	0.63	0.30	0.41	0.13	1.24	0.08
5	0.41	0.18	0.18	0.06	1.28	0.29
6	0.15	0.06	0.53	0.41	0.95	0.15
7	0.07	0.03	0.90	0.57	0.84	0.31
9	0.17	0.11	0.73	0.46	0.80	0.31
12	0.06	0.06	1.21	0.37	0.70	0.08
13	0.02	0.01	1.01	0.43	0.95	0.47
14	0.05	0.04	1.57	0.45	0.27	0.00
15	0.07	0.05	2.18	0.15	0.37	0.15
18	0.06	0.02	2.31	0.24	0.75	0.23
19	0.10	0.02	2.22	0.10	0.32	0.01
20	0.00	0.00	0.89	0.15	1.40	0.07
22	0.00	0.01	0.02	0.01	2.22	0.20
24	0.02	0.01	0.02	0.01	2.24	0.16
25	0.01	0.01	0.02	0.01	2.16	0.13
27	0.01	0.01	0.01	0.00	2.07	0.24
28	0.01	0.00	0.01	0.00	2.11	0.14

Table C-2 Concentration of ammonia, nitrite and nitrate in water column from the treatment-1 which packed with air-dried sediment.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.34	0.13	0.13	0.09	1.04	0.29
2	0.27	0.03	0.18	0.09	0.67	0.20
5	0.05	0.02	0.16	0.08	1.14	0.40
6	0.07	0.04	0.68	0.06	0.74	0.41
7	0.06	0.03	0.77	0.39	0.87	0.14
8	0.10	0.05	0.42	0.17	1.12	0.21
11	0.09	0.05	0.07	0.05	1.32	0.25
12	0.06	0.01	0.04	0.03	1.27	0.16
14	0.00	0.00	0.05	0.02	1.34	0.17
15	0.01	0.01	0.02	0.01	1.31	0.11
17	0.03	0.02	0.02	0.01	1.57	0.33
18	0.02	0.01	0.02	0.01	1.33	0.09
20	0.04	0.02	0.02	0.02	1.39	0.11
21	0.01	0.01	0.01	0.00	1.39	0.22
22	0.02	0.01	0.01	0.00	1.19	0.05
23	0.03	0.02	0.02	0.01	1.06	0.05
25	0.09	0.05	0.03	0.01	1.36	0.08
26	0.00	0.00	0.01	0.00	1.23	0.03
27	0.00	0.00	0.02	0.00	1.48	0.12
28	0.03	0.06	0.01	0.00	1.22	0.01

Table C-3 Concentration of ammonia, nitrite and nitrate in water column from the treatment-2 which packed with sunlight-dried sediment.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.97	0.31	0.05	0.03	1.17	0.60
2	1.69	0.10	0.29	0.14	0.71	0.18
5	0.74	0.24	0.65	0.18	1.03	0.20
6	0.67	0.31	0.38	0.18	1.85	0.14
7	0.20	0.06	0.72	0.45	2.10	0.13
8	0.15	0.08	0.79	0.56	2.28	0.49
11	0.08	0.04	2.06	0.36	2.78	0.44
12	0.06	0.02	0.44	0.14	2.40	0.27
14	0.00	0.00	0.30	0.08	3.14	0.76
15	0.02	0.01	0.06	0.04	3.08	0.61
17	0.03	0.01	0.04	0.01	3.20	0.71
18	0.02	0.01	0.02	0.01	3.32	0.65
20	0.04	0.02	0.03	0.01	3.24	0.71
21	0.03	0.02	0.02	0.01	3.64	0.81
22	0.03	0.01	0.02	0.01	2.79	0.30
23	0.05	0.02	0.04	0.02	2.47	0.29
25	0.07	0.03	0.08	0.11	3.16	0.46
26	0.00	0.00	0.02	0.01	3.03	1.09
27	0.00	0.00	0.01	0.00	3.13	0.87
28	0.00	0.00	0.01	0.00	3.07	0.71

Table C-4 Concentration of ammonia, nitrite and nitrate in pore water from the control chamber packed with untreated wet sediment.

Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	1.13	0.13	0.08	0.01	3.52	0.25
7	9.07	0.15	3.85	0.14	17.77	0.22
14	1.57	0.13	3.35	0.12	15.45	0.20
21	38.08	0.28	1.21	0.12	23.10	0.34
28	2.46	2.09	0.00	0.01	8.08	0.29

Table C-5 Concentration of ammonia, nitrite and nitrate in pore water from the treatment-1 which packed with air-dried sediment.

Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	10.74	0.36	0.08	0.04	20.55	0.09
7	2.56	0.65	0.12	0.06	45.29	0.30
14	2.12	0.58	1.21	0.16	34.97	4.88
21	9.68	0.10	0.13	0.11	3.43	0.09
28	40.80	0.10	0.38	0.12	14.80	0.29

Table C-6 Concentration of ammonia, nitrite and nitrate in pore water from the treatment-2 which packed with sunlight-dried sediment.

Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	7.85	0.30	0.91	0.37	37.45	0.61
7	14.28	0.28	0.49	0.27	11.46	0.42
14	8.40	0.43	1.26	0.44	15.85	0.51
21	2.41	0.16	0.36	0.09	3.68	0.04
28	19.70	0.12	1.38	0.46	9.52	0.79

Table C-7 Concentration of ammonia, nitrite and nitrate in extracted soil from the control chamber packed with untreated wet sediment.

Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	10.61	1.30	0.07	0.00	1.73	0.11
7	4.31	0.36	0.12	0.16	3.70	0.06
14	8.19	1.31	0.15	0.21	4.84	0.08
21	6.96	0.02	0.45	1.08	9.63	0.22
28	4.13	0.11	0.17	0.14	4.90	0.03

Table C-8 Concentration of ammonia, nitrite and nitrate in extracted soil from the treatment-1 which packed with air-dried sediment.

Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	18.48	1.97	0.19	0.09	4.77	0.32
7	4.52	0.34	0.17	0.17	9.51	0.26
14	7.04	0.54	0.24	0.10	11.40	0.20
21	3.85	0.45	0.23	0.10	7.29	2.40
28	8.79	0.35	0.44	0.40	12.12	0.32

Table C-9 Concentration of ammonia, nitrite and nitrate in extracted sediment from the treatment-2 which packed with sunlight-dried sediment.

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Day	Ammonia (mg-N/g wet sediment)		Nitrite (mg-N/g wet sediment)		Nitrate (mg-N/g wet sediment)	
	Average	SD	Average	SD	Average	SD
0	49.07	7.34	0.50	0.11	4.25	0.23
7	5.13	0.68	0.34	0.42	8.66	0.24
14	9.84	0.14	0.19	0.04	12.81	0.49
21	6.40	0.26	0.30	0.39	4.78	0.17
28	8.90	0.13	0.24	0.19	7.72	0.36

Table C-10 Concentration of ammonia, nitrite and nitrate in water column the untreated wet sediment and no ammonium chloride addition.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.38	0.10	0.02	0.00	1.62	0.07
1	0.46	0.10	0.04	0.01	1.10	0.06
2	0.32	0.17	0.11	0.01	1.00	0.19
3	0.21	0.10	0.15	0.03	1.20	0.01
4	0.25	0.16	0.15	0.05	1.21	0.04
5	0.19	0.12	0.10	0.03	1.31	0.06
6	0.13	0.10	0.07	0.03	1.43	0.12
7	0.11	0.10	0.05	0.03	1.39	0.03
8	0.12	0.07	0.05	0.05	1.55	0.06
9	0.15	0.10	0.07	0.06	1.58	0.04
10	0.08	0.06	0.08	0.10	1.25	0.49
11	0.05	0.05	0.03	0.02	1.62	0.03
12	0.06	0.05	0.04	0.04	1.64	0.48
14	0.06	0.06	0.04	0.05	1.48	0.03
16	0.01	0.01	0.11	0.14	1.48	0.12
18	0.00	0.00	0.15	0.20	1.71	0.05
20	0.01	0.02	0.04	0.03	1.74	0.08
22	0.03	0.02	0.01	0.00	1.27	0.98
24	0.02	0.01	0.01	0.01	1.82	0.00
26	0.00	0.00	0.02	0.01	1.87	0.09

Table C-11 Concentration of ammonia, nitrite and nitrate in water column the untreated wet sediment and with ammonium chloride addition (2 mg-N/L).

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	2.17	0.38	0.02	0.00	1.53	0.01
1	2.24	0.17	0.06	0.01	1.15	0.01
2	1.52	0.19	0.31	0.04	1.00	0.00
3	1.08	0.22	0.57	0.04	1.04	0.02
4	0.78	0.07	0.83	0.03	0.91	0.05
5	0.36	0.02	0.83	0.05	1.23	0.02
6	0.13	0.02	0.61	0.07	1.47	0.03
7	0.05	0.01	0.35	0.05	1.67	0.00
8	0.05	0.01	0.18	0.06	1.84	0.03
9	0.06	0.01	0.09	0.03	1.98	0.08
10	0.06	0.02	0.04	0.01	1.81	0.38
11	0.05	0.06	0.03	0.01	2.05	0.01
12	0.05	0.06	0.03	0.02	1.98	0.14
14	0.05	0.04	0.03	0.02	1.72	0.12
16	0.03	0.04	0.02	0.01	1.90	0.21
18	0.00	0.00	0.01	0.00	2.07	0.16
20	0.00	0.00	0.02	0.01	1.71	0.19
22	0.02	0.01	0.01	0.00	1.98	0.22
24	0.02	0.01	0.01	0.01	1.94	0.08
26	0.00	0.01	0.01	0.00	1.89	0.18

Table C-12 Concentration of ammonia, nitrite and nitrate in water column the sunlight-dried sediment and no ammonium chloride addition.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.51	0.08	0.02	0.00	1.84	0.03
1	1.15	0.20	0.06	0.01	1.51	0.03
2	1.35	0.09	0.08	0.01	1.51	0.20
3	2.21	0.33	0.11	0.03	1.60	0.09
4	5.02	0.62	0.16	0.03	1.47	0.00
5	3.03	0.21	0.19	0.04	1.53	0.07
6	3.32	0.37	0.34	0.07	1.47	0.06
7	3.29	0.30	0.56	0.16	1.38	0.03
8	3.24	0.30	1.02	0.23	1.34	0.12
9	2.26	0.13	2.10	0.20	1.06	0.10
10	1.05	0.16	2.96	0.37	1.45	0.23
11	0.38	0.13	4.03	0.86	2.72	0.48
12	0.08	0.05	4.67	0.56	0.71	0.17
14	0.03	0.02	5.28	0.71	0.10	0.44
16	0.01	0.02	4.16	0.65	1.00	0.19
18	0.00	0.00	2.55	0.67	3.11	0.38
20	0.01	0.01	0.70	0.77	4.73	0.08
22	0.03	0.02	0.05	0.03	4.07	0.80
24	0.02	0.01	0.01	0.00	5.59	0.44
26	0.01	0.01	0.01	0.01	5.46	0.36

Table C-13 Concentration of ammonia, nitrite and nitrate in water column the sunlight-dried sediment and with ammonium chloride addition (2 mg-N/L).

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	2.42	0.19	0.02	0.00	2.01	0.34
1	4.40	0.30	0.06	0.01	1.37	0.00
2	3.81	0.33	0.08	0.01	1.46	0.02
3	4.59	0.72	0.10	0.01	1.52	0.00
4	3.10	0.32	0.13	0.03	1.46	0.06
5	5.04	0.42	0.60	0.05	1.15	0.01
6	4.84	0.61	0.50	0.13	1.45	0.02
7	4.26	0.51	0.89	0.26	1.30	0.07
8	4.54	0.83	1.70	0.61	1.24	0.10
9	1.19	0.63	3.68	1.17	0.71	0.04
10	1.53	1.00	5.15	0.67	2.17	0.04
11	0.38	0.24	5.23	0.43	0.72	0.05
12	0.12	0.09	5.87	0.75	0.44	0.13
14	0.02	0.01	6.80	0.63	0.12	0.14
16	0.00	0.01	5.91	0.99	0.58	0.49
18	0.00	0.00	4.04	1.88	2.42	1.41
20	0.01	0.01	2.23	1.96	4.12	1.24
22	0.03	0.01	0.46	0.55	5.76	0.15
24	0.01	0.01	0.01	0.00	6.25	0.56
26	0.00	0.01	0.02	0.00	5.96	0.32

Table C-14 Concentration of ammonia, nitrite and nitrate in water column from the control tank packed with untreated wet sediment.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.03	0.02	0.01	0.00	1.23	0.07
1	0.06	0.05	0.01	0.00	1.07	0.16
2	0.07	0.02	0.01	0.00	1.22	0.04
3	0.05	0.02	0.01	0.00	1.17	0.03
4	0.02	0.02	0.01	0.02	1.10	0.05
5	0.00	0.01	0.00	0.00	1.22	0.07
6	0.00	0.00	0.01	0.00	1.22	0.14
7	0.02	0.01	0.01	0.00	1.16	0.11
8	0.00	0.00	0.02	0.01	0.98	0.19
9	0.00	0.00	0.00	0.00	0.94	0.17
10	0.05	0.04	0.02	0.02	0.80	0.17
11	0.08	0.04	0.01	0.01	0.76	0.02
12	0.05	0.03	0.01	0.01	0.68	0.03
12	2.39	0.19	0.00	0.00	0.68	0.03
13	1.88	0.11	0.07	0.07	1.00	0.37
14	1.88	0.11	0.07	0.08	1.01	0.37
15	1.76	0.29	0.03	0.01	0.88	0.27
16	1.80	0.38	0.03	0.01	0.85	0.01
17	1.25	0.20	0.05	0.01	0.93	0.10
18	0.81	0.11	0.07	0.00	1.00	0.12
19	0.63	0.11	0.07	0.00	1.03	0.12
20	0.61	0.12	0.07	0.01	1.14	0.15
21	0.44	0.10	0.11	0.02	1.28	0.15
22	0.37	0.11	0.11	0.04	1.32	0.16
23	0.20	0.13	0.12	0.04	1.45	0.19
24	0.16	0.08	0.14	0.01	1.29	0.04
25	0.14	0.08	0.09	0.04	1.46	0.11
26	0.08	0.05	0.08	0.04	1.52	0.07

Table C-15 Concentration of ammonia, nitrite and nitrate in water column from the treatment tank packed with sun-dried sediment.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.14	0.15	0.02	0.00	1.37	0.10
1	0.24	0.13	0.03	0.01	1.43	0.17
2	0.29	0.19	0.04	0.02	1.52	0.11
3	0.35	0.22	0.05	0.03	1.46	0.17
4	0.21	0.20	0.05	0.06	1.53	0.08
5	0.21	0.23	0.08	0.09	1.59	0.10
6	0.17	0.19	0.14	0.14	1.68	0.02
7	0.17	0.18	0.17	0.18	1.76	0.06
8	0.06	0.07	0.19	0.20	1.77	0.14
9	0.01	0.01	0.15	0.17	1.66	0.21
10	0.02	0.01	0.17	0.18	1.54	0.07
11	0.05	0.04	0.09	0.12	1.14	0.36
12	0.05	0.04	0.09	0.09	1.14	0.21
12	2.53	0.15	0.04	0.05	1.14	0.21
13	1.97	0.32	0.01	0.01	1.03	0.34
14	1.91	0.34	0.01	0.01	1.04	0.36
15	1.31	0.16	0.20	0.03	1.38	0.20
16	1.11	0.11	0.30	0.05	1.54	0.18
17	0.64	0.17	0.37	0.03	1.76	0.13
18	0.32	0.17	0.31	0.04	1.96	0.09
19	0.15	0.17	0.27	0.09	2.02	0.10
20	0.23	0.18	0.22	0.15	2.11	0.18
21	0.11	0.13	0.19	0.16	1.96	0.53
22	0.07	0.08	0.16	0.16	2.14	0.36
23	0.04	0.04	0.16	0.12	2.18	0.32
24	0.03	0.02	0.11	0.09	1.73	0.72
25	0.00	0.01	0.07	0.06	2.16	0.45
26	0.02	0.01	0.03	0.02	2.15	0.52

Table C-16 Concentration of ammonia, nitrite and nitrate in water column from the control tank packed with sun-dried sediment and supplemented with ammonia at 0.5 mg-N/L/day.

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Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.41	0.02	0.01	0.00	1.08	0.17
0	0.28	0.02	0.00	0.00	1.05	0.19
1	0.72	0.03	0.03	0.01	0.92	0.13
1	0.64	0.04	0.03	0.01	1.10	0.13
2	0.95	0.05	0.08	0.01	1.15	0.13
2	0.83	0.03	0.08	0.02	1.04	0.08
3	1.04	0.03	0.13	0.02	1.18	0.08
3	1.06	0.05	0.15	0.01	1.28	0.16
4	0.84	0.06	0.14	0.01	0.48	0.01
4	0.67	0.09	0.14	0.01	1.17	0.07
5	0.88	0.08	0.19	0.02	1.04	0.24
5	1.41	0.35	0.20	0.02	1.75	0.00
6	1.40	0.05	0.34	0.04	1.82	0.15
6	0.98	0.05	0.29	0.03	1.79	0.04

Table C-17 Concentration of ammonia, nitrite and nitrate in water column from the treatment tank packed with sun-dried sediment and supplemented with ammonia at 0.5 mg-N/L/day.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.34	0.04	0.01	0.00	1.48	0.57
0	0.15	0.16	0.00	0.00	1.47	0.57
1	0.58	0.24	0.02	0.02	1.38	0.57
1	0.38	0.33	0.02	0.02	1.52	0.59
2	0.73	0.34	0.06	0.04	1.53	0.68
2	0.55	0.46	0.06	0.06	1.45	0.56
3	0.85	0.46	0.09	0.09	1.58	0.70
3	0.75	0.56	0.09	0.09	1.56	0.60
4	0.72	0.40	0.11	0.10	0.58	0.28
4	0.58	0.37	0.12	0.10	1.26	0.56
5	0.81	0.36	0.15	0.15	1.13	0.56
5	0.60	0.19	0.11	0.11	1.36	0.44
6	1.51	0.29	0.46	0.44	1.62	0.84
6	1.08	0.13	0.50	0.48	1.40	0.70

Table C-18 Concentration of ammonia, nitrite and nitrate in water column from the control tank packed with sun-dried sediment and supplemented with ammonia at 0.2 mg-N/L/day.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.07	0.01	0.12	0.02	0.19	0.01
0	0.26	0.05	0.22	0.04	3.12	0.22
1	0.20	0.06	0.14	0.02	2.02	0.12
1	0.20	0.09	0.11	0.01	2.11	0.09
2	0.26	0.04	0.10	0.02	2.24	0.09
2	0.30	0.03	0.11	0.01	2.11	0.08
3	0.33	0.04	0.12	0.01	2.74	0.11
3	0.46	0.09	0.11	0.02	2.73	0.14
4	0.11	0.08	0.12	0.01	2.63	0.10
4	0.35	0.09	0.10	0.02	2.92	0.14
5	0.31	0.06	0.11	0.03	3.08	0.08
5	0.47	0.04	0.13	0.03	3.42	0.09
6	0.23	0.13	0.10	0.02	3.17	0.19
6	0.54	0.06	0.14	0.03	3.68	0.04
7	0.35	0.02	0.12	0.04	3.20	0.10
7	0.51	0.03	0.12	0.03	3.10	0.11
8	0.53	0.06	0.15	0.03	3.23	0.09
8	0.35	0.02	0.12	0.03	2.85	0.07
9	0.37	0.03	0.13	0.04	3.33	0.10
9	0.50	0.04	0.17	0.04	3.73	0.14
10	0.47	0.04	0.19	0.04	4.60	0.16
10	0.39	0.04	0.15	0.04	2.86	0.01
11	0.34	0.03	0.13	0.05	2.83	0.05
11	0.36	0.12	0.14	0.04	3.67	0.05
12	0.47	0.03	0.20	0.05	2.97	0.06
12	0.35	0.03	0.16	0.05	3.76	0.08
13	0.24	0.02	0.17	0.06	3.63	0.05
13	0.34	0.18	0.16	0.06	3.80	0.05

Table C-19 Concentration of ammonia, nitrite and nitrate in water column from the treatment tank packed with sun-dried sediment and supplemented with ammonia at 0.2 mg-N/L/day.

Day	Ammonia (mg-N/L)		Nitrite (mg-N/L)		Nitrate (mg-N/L)	
	Average	SD	Average	SD	Average	SD
0	0.50	0.40	0.77	0.57	2.25	1.77
0	0.33	0.17	0.37	0.30	1.78	1.14
1	0.31	0.22	0.35	0.27	1.83	1.17
1	0.41	0.19	0.32	0.26	1.96	1.19
2	0.43	0.20	0.29	0.22	1.82	1.23
2	0.42	0.18	0.30	0.22	2.36	1.32
3	0.69	0.31	0.29	0.20	2.44	1.55
3	0.39	0.25	0.29	0.19	2.51	1.48
4	0.59	0.27	0.26	0.16	2.64	1.64
4	0.55	0.31	0.24	0.14	2.73	1.66
5	0.70	0.33	0.27	0.15	3.07	1.99
5	0.42	0.27	0.22	0.10	2.74	1.71
6	0.89	0.29	0.24	0.09	3.36	2.10
6	0.64	0.37	0.21	0.08	2.82	1.75
7	0.85	0.42	0.22	0.05	2.76	1.84
7	0.85	0.34	0.22	0.05	2.84	1.83
8	0.66	0.36	0.18	0.02	2.56	1.56
8	0.69	0.33	0.22	0.05	3.45	2.31
9	0.93	0.47	0.23	0.02	3.45	2.34
9	0.92	0.54	0.21	0.01	4.06	2.62
10	0.71	0.42	0.20	0.01	2.63	1.91
10	0.69	0.39	0.17	0.02	2.52	1.89
11	0.73	0.44	0.20	0.03	3.36	1.88
11	0.89	0.53	0.26	0.03	2.55	1.68
12	0.87	0.53	0.20	0.05	3.47	1.94
12	0.63	0.46	0.21	0.05	3.24	1.99
13	0.83	0.48	0.21	0.07	3.42	1.90
13	0.71	0.42	0.18	0.08	3.22	1.93

APPENDIX D

GENBANK FLATE FILE OF SEQUENCES AMPLIFIED FROM BACTERIA AND EUKARYOTIC PLANKTON

```
LOCUS       FJ665399              173 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665399
VERSION     FJ665399
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 173)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 173)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..173
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-1"
                     /environmental_sample
     rRNA            <1..>173
                     /product="16S ribosomal RNA"
ORIGIN
        1 ctcctacggg aggcagcagt ggggaatatt ggacaatggg ggaaaccctg atccagccat
       61 gccgcgtgtg tgaagaaggc ccgagggttg taaagcactt tncagttggg aaaaagggta
      121 gtaagttaat anccttgcta tncttgatat taccttcaga agaagccccg tat
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```
LOCUS       FJ665400              173 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-2 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665400
VERSION     FJ665400
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 173)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 173)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..173
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-2"
                     /environmental_sample
     rRNA            <1..>173
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcctacgg gaggcagcag tggggaatat tggacaatgg gcgaaagcct gatccagcca
       61 tgccgcgtgt gtgaagaagg tcttcggatt gtaaagcact ttaagttggg aggaagagca
      121 ttaacctaat ancgttagtg ttttgacgtt accgaccaga ataagccccg tat
//


LOCUS       FJ665401              170 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665401
VERSION     FJ665401
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 170)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 170)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..170
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-3"
                     /environmental_sample
     rRNA            <1..>170
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcctacgg gatggcagca gtggggaata ttggacaatg ggcgaaagcc tgatccagcc
       61 atgccgcgtg tgtgaagaag gtcttcggat tgtaaagcac tttaagttgg gaggaagggc
      121 attaacctaa tacgttagtg tttgacgtac cgacagaaaa gccccgnatg
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LOCUS       FJ665402              175 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-4 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665402
VERSION     FJ665402
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 175)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 175)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..175
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-4"
                     /environmental_sample
     rRNA            <1..>175
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcntacgg gaggcagcag tggggaatat tggacaatgg gcgcaagcct gatccagcca
       61 tgccgcgtgt gtgaagaagg ccttcgggtt gtaaagcact ttcagcgagg aggaagggtg
      121 gtgtgttaat agccagcatg cattttgacc gtaacncgca gaagaagctc cgtat
//


LOCUS       FJ665403              182 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-5 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665403
VERSION     FJ665403
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 182)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 182)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..182
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-5"
                     /environmental_sample
     rRNA            <1..>182
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcctacgg gaggcagcag tagggaatct tccgcaatgg acgaaagtct gacggagcaa
       61 cgccgcgtga gcgatgaagg ccttcgggtc gtaaagctac tgttgtncag ggaagaanca
      121 agtatacgga gtaagctgnc cggtaccctt gaacgntanc nganccagaa aggccccgta
      181 tc
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LOCUS       FJ665404              178 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-6 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665404
VERSION     FJ665404
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 178)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 178)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..178
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-6"
                     /environmental_sample
     rRNA            <1..>178
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcctacgg gaggcagcag tggggaatat tggacaatgg gggcaaccct gatccagcca
       61 tgccgcgtgt gtgaagaagg ccctacgggt tgtaaagcac ttttcagccg aggaagaacg
      121 acctggtggt tnatagcccg accaggaaag aactncatcc cgcagaagaa gccccgta
//


LOCUS       FJ665405              172 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone BAC-7 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665405
VERSION     FJ665405
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 172)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Bacterial community in sediment from shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 172)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..172
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="BAC-7"
                     /environmental_sample
     rRNA            <1..>172
                     /product="16S ribosomal RNA"
ORIGIN
        1 actcctacgg gaggcagcag tggggaatat tgcacaatgg gcgcaagcct gatgcagcca
       61 tgccgcgtgt atgaagaagg ccttcgggtt gtaaagtact ttncagcggg gaggaaggga
      121 gtaaagttaa taccttgct cattgacgta cccgcagaag aagccccgta tc
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LOCUS       FJ665406              161 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665406
VERSION     FJ665406
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 161)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 161)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..161
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-1"
                     /environmental_sample
     rRNA            <1..>161
                     /product="16S ribosomal RNA"
ORIGIN
        1 tatcgagcgc aatatttgcg aaccaatggg cgaaagcctg atgcagcaac gccgcgtgcg
       61 cgaagaaggc cttcgggctg taaagcgctt ttctgggaga tgaaaaagga cagtatccca
      121 cgaataagtc tcggctaact acgtgccagc agccccggtt a
//

LOCUS       FJ386437              184 bp    DNA     linear   ENV 05-NOV-2008
DEFINITION  Uncultured bacterium isolate DGGE gel band ORG-2 16S ribosomal
            RNA gene, partial sequence.
ACCESSION   FJ386437
VERSION     FJ386437
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 184)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            diversity in sediment from shrimp pond under laboratory
            conditions
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 184)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-OCT-2008) Environmental Science, Chulalongkorn
            University, Bangkok 10330, Thailand
COMMENT     LocalID: bankit1143208
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolate="DGGE gel band B-2"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /environmental_sample
     rRNA            <1..>184
                     /product="16S ribosomal RNA"
ORIGIN
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       61 gaagaaggcc tgcgggttgt aaagcacttt ctttgggaac gaaatatgcc gacctagtac
      121 gtcggtaagc tgacgttacc caaataaaaa gcaccggcta actctgtgcc cgcagccgcg
      181 ataa
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```
LOCUS       FJ665407              180 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665407
VERSION     FJ665407
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 180)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 180)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
             Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..180
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-3"
                     /environmental_sample
     rRNA            <1..>180
                     /product="16S ribosomal RNA"
ORIGIN
        1 cgaggcgtga atattgcaca atgggcgcaa gcctgatgca gccatgccgc gtgtatgaag
       61 aaggccttcg ggttgtaaag tactttcagc agtgaggaag gcggatgcgt taatagcgta
      121 ttcgtttgac gttagctgca gaagaagcac cggctaactc cgtgccagca gccgcggtaa
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LOCUS       FJ386438              158 bp    DNA     linear   ENV 05-NOV-2008
DEFINITION  Uncultured bacterium isolate DGGE gel band ORG-4 16S ribosomal
            RNA gene, partial sequence.
ACCESSION   FJ386438
VERSION     FJ386438
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 158)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            diversity in sediment from shrimp pond under laboratory
            conditions
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 158)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-OCT-2008) Environmental Science, Chulalongkorn
            University, Bangkok 10330, Thailand
COMMENT     LocalID: bankit1143277
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /isolate="DGGE gel band B-4"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /environmental_sample
     rRNA            <1..>158
                     /product="16S ribosomal RNA"
ORIGIN
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       61 gaaggtcttc ggatcgtaaa gctctgtcct tagggaagaa tattgacggt acctaaggag
      121 gaagccccgg ctaactacgt gccagcagcc gcggtaaa
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LOCUS       FJ665408              176 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-5 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665408
VERSION     FJ665408
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 176)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 176)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
             Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-5"
                     /environmental_sample
     rRNA            <1..>176
                     /product="16S ribosomal RNA"
ORIGIN
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       61 gaaggcctgc gggttgtaaa gcactttccg ttgggaagaa aaaagacggg cccataccgg
      121 cgggtcttga cgttacctac gcaagaagca ccggctaaat ccgtgccagc agccgc
//


LOCUS       FJ665409              156 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-6 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665409
VERSION     FJ665409
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 156)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 156)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
             Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..156
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-6"
                     /environmental_sample
     rRNA            <1..>156
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgtgcgtgaa tattgcacaa tgggggaaac cctgatgcag cgacgccgcg tgagcgatga
       61 aggccttcgg gtcgtaaagc tctgtcctat gggaagaata aatgacggta ccataggagg
      121 aagccccggc taactacgtg ccagcagccg cggtaa
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LOCUS       FJ665410              183 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-7 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665410
VERSION     FJ665410
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 183)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 183)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-7"
                     /environmental_sample
     rRNA            <1..>183
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggcgggcgt aatatttgcg acaatggggg aaaccctgat ccagcgacgc cgcgtgggtg
       61 aagaaggcct gcgggttgta aagccctttc ggtagggacg aaagctctcg acctaacacg
      121 tcgggaggtt gacttatcct tgccaagaag ccccggctaa atctgtgccc gcagccgcgg
      181 taa
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LOCUS       FJ665411              180 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-8 16S ribosomal RNA gene, partial sequence.
ACCESSION   FJ665411
VERSION     FJ665411
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 180)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 180)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..180
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-8"
                     /environmental_sample
     rRNA            <1..>180
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggcgtgcga atattggaca atgggcgaaa gcctgatcca gccatgccgc gtgtgtgaag
       61 aaggtcttcg gattgtaaag cactttaagt tgggaggaag ggcagtaagt taataccttg
      121 ctgttttgac gttaccgaca gaataagcac cggctaactt cgtgccagca gccgcggtaa
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LOCUS       FJ665412              179 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-9 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665412
VERSION     FJ665412
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-9"
                     /environmental_sample
     rRNA            <1..>179
                     /product="16S ribosomal RNA"
ORIGIN
        1 tacgggcgca atcttccgca atggacgaaa gtctgacgga gcaacgccgc gtgagtgaag
       61 aaggttttcg gatcgtaaaa ctctgttgtg agggaagaac aagtaccaac taactactgg
      121 taccttgacg gtacctcacc agaaagccac ggctaactac gtgccagcag ccgcggtaa
//


LOCUS       FJ665413              160 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-10 16S ribosomal RNA gene,
              partial sequence.
ACCESSION   FJ665413
VERSION     FJ665413
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 160)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 160)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
             University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..160
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-10"
                     /environmental_sample
     rRNA            <1..>160
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgggaaaggt gcaatatttg cgacaatggg cggaaagcct gatccagcca cgccgcgtga
       61 gggatgaagg ccttcgggtt gtaaacctct ttttttggg aagaaaaagg gcgggcctat
      121 aggccaaagc ccgggccgtt cccttaccag aagccccggt
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LOCUS       FJ665414              155 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-11 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   FJ665414
VERSION     FJ665414
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..155
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-11"
                     /environmental_sample
     rRNA            <1..>155
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgggggggcg caatatttgc ggcaatgggc gaaagcctga tgcagccacg ccgcgtgcgg
       61 gatgaaggcc ttcgggttgt aaacctcttt tcttggggac gaagaaggac ggtccctccc
      121 gactaagtct cggctaactc cctgcctgcc gcccc
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LOCUS       FJ665415              185 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-12 16S ribosomal RNA gene,partial sequence.
ACCESSION   FJ665415
VERSION     FJ665415
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 185)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 185)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-12"
                     /environmental_sample
     rRNA            <1..>185
                     /product="16S ribosomal RNA"
ORIGIN
        1 ccttaggggg gagaaatatt ggacaatagg gcgaaagcct gatccagcca tgccgcgtgt
       61 gtgaagaagg tcttcggatt gtaaagcact ttaagttggg aggaagggtt gtagtttaat
      121 acgctgcaat cttgacgtta ccaacagaat aagcaccggc taactctgtg ccagcagccg
      181 cggta
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LOCUS       FJ665416             156 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-13 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   FJ665416
VERSION     FJ665416
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 156)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 156)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..156
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-13"
                     /environmental_sample
     rRNA            <1..>156
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggtgggggg aatttttgcg caatgggcga aagcctgacg cagcgacgcc gcgtggagga
       61 tgaaggtctt cggattgtaa actccttttg caggggaaaa taatgatggt accctgcgaa
      121 taagccacgg ctaactctgt gccagcagcc gcggta
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LOCUS       FJ665417             155 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-14 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   FJ665417
VERSION     FJ665417
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-14"
                     /environmental_sample
     rRNA            <1..>155
                     /product="16S ribosomal RNA"
ORIGIN
        1 aacgcgagaa tattggcaca atgggcgaaa gcctgatgca gcaacgccgc gtgcgcgatg
       61 aagaccttcg ggttgtaaag cgcttttctg aaggacgagg aaggacggta cttcaggaat
      121 aaggatcggc taactacgtg ccagcagccg cggta
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LOCUS       FJ665418              175 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-15 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   FJ665418
VERSION     FJ665418
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 175)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory
            condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 175)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-15"
                     /environmental_sample
     rRNA            <1..>175
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgcacggaat attaggacaa tgggggaaac gcctgatcca gccatgccgc gtgcgggaag
       61 actgccctat gggttgtaaa ctgcttttat tggggaagaa acgtacctac gtgtaggtgc
      121 ttgacgggac cgtacgaata aagatcggct aactccatgc cagcacccgc ggtaa
//


LOCUS       FJ665419              185 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone ORG-16 16S ribosomal RNA gene,partial sequence.
ACCESSION   FJ665419
VERSION     FJ665419
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 185)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Effect of methanol addition on nitrogen conversion and bacterial
            community in sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 185)
  AUTHORS   Kutako,M., Limpiyakorn,T., Luepromchai,E., Powtongsook,S. and
            Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2008) Graduate School, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="ORG-16"
                     /environmental_sample
     rRNA            <1..>185
                     /product="16S ribosomal RNA"
ORIGIN
        1 ttgtacgcga atattagctg caatgggcgg agagcctgac ccagccatgc cgcgtgcagg
       61 aagaaggccc tacggtttgt aaactgtttt tctggggaag aaaatgagag tacccgtaaa
      121 cgattgccgg gatcatgcga agaaacctcg gctagctctg tgccagcagc cgcggtaatt
      181 aataa
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LOCUS       FJ595662              181 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595662
VERSION     FJ595662
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in sediment
            from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn University,
            254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
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                     /db_xref="taxon:77133"
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                     /environmental_sample
     rRNA            <1..>181
                     /product="16S ribosomal RNA"
ORIGIN
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       61 aggtcttcgg attgtaaagc tctgttatta gggaagaaag aaattagtag gaaatgacta
      121 ataagtgacg gtacctaatg agaaagccac ggctaactac gtgccagcag ccgcggtaat
      181 t
//


LOCUS       FJ595663              180 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-2 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595663
VERSION     FJ595663
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 180)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 180)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
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                     /db_xref="taxon:77133"
                     /clone="DES-2"
                     /environmental_sample
     rRNA            <1..>180
                     /product="16S ribosomal RNA"
ORIGIN
        1 aaagagtgaa tcttcggcaa tggacgaaag tctgaccgag caacgccgcg tgagtgaaga
       61 aggtcttcgg attgtaaagc tctgttatta gggaagaaag agattagtag gaaatgacta
      121 ataagtgacg gtacctaatg agaaagccac ggctaactac gtgccagcag ccgcggtaat
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LOCUS       FJ595664              156 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595664
VERSION     FJ595664
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 156)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 156)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..156
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-3"
                     /environmental_sample
     rRNA            <1..>156
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggcgcgaga atattggcac aatgggggaa accctgatgc agcaacgccg cgtggaggat
       61 gacgcatttc ggtgtgtaaa ctccttttat ataggaagat aatgacggta ctatatgaat
      121 aagcaccggc taactccgtg ccagcagccg cggtaa
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LOCUS       FJ595665              159 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-4 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595665
VERSION     FJ595665
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 159)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in sediment
            from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 159)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University,  254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..159
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-4"
                     /environmental_sample
     rRNA            <1..>159
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggtggtggg aatattggca caatgggggg gaaccctgat gcagcaacgc cgcgtggagg
       61 atgacacatt tcggtgcgta aactcctttt atataggaag ataatgacgg tactatatga
      121 ataagcaccg gctaactccg tgccagcagc cgcggtaat
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LOCUS       FJ595666              181 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-5 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595666
VERSION     FJ595666
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..181
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-5"
                     /environmental_sample
     rRNA            <1..>181
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggcggggga attttggaca atgggcgcaa gcctgatcca gcaatgccgc gtgagtgaag
       61 aaggccttcg ggttgtaaag ctctttcagt tgagaagaaa aggttgtggt aaataatcac
      121 aattgatgac ggtatcgaca gaagaagcac cggctaacta cgtgccagca gccgcgataa
      181 t
//


LOCUS       FJ595667              181 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-6 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595667
VERSION     FJ595667
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..181
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-6"
                     /environmental_sample
     rRNA            <1..>181
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggaacggaa ttattagcga caatgggcga aagcctgatc cagcaatacc gcgtgtgtga
       61 agaaggcctg agggttgtaa agcactttca atagggagga atacctaccg gttaataccc
      121 ggtagactga cattgcctat acgaaaagca cccgctaact ccctgccccc ccccccggta
      181 a
//
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```
LOCUS       FJ595668              181 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-7 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595668
VERSION     FJ595668
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn University,
            254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..181
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-7"
                     /environmental_sample
     rRNA            <1..>181
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgtgggcgaa tattagcgac aatgggggaa accctgatcc agcaatgccg cgtgtgtgaa
       61 gaaggccttc gggttgtaaa gcactttcaa tagggaggaa aagctatgcg ttaatagcgt
      121 atagccgtga cgttacctat agaagaagca ccggctaact ccgtgccagc agccgcggta
      181 a
//


LOCUS       FJ595669              180 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-8 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595669
VERSION     FJ595669
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 180)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 180)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..180
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-8"
                     /environmental_sample
     rRNA            <1..>180
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgcgcgtgaa tattggacaa tgggggcaac cctgatccag caatgccgcg tgtgtgaaga
       61 aggcctgagg gttgtaaagc actttcagta gtgaggaaaa gtttacggtt aatacccgtg
      121 aaccctgacg ttaactacag aagaagcacc ggctaactcc gtgccagcag ccgcggtaat
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LOCUS       FJ595670              173 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-9 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ595670
VERSION     FJ595670
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 173)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in
            Sediment from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 173)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..173
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-9"
                     /environmental_sample
     rRNA            <1..>173
                     /product="16S ribosomal RNA"
ORIGIN
        1 tggtaggcaa tattggtcaa tgggcgcgag cctgaaccag ccatcccgcg tgcaggatga
       61 cggccctatg ggttgtaaac tgcttttcta ctccaagaaa ccaccttacg tgtagggtgt
      121 tgccggtaga gtaggaataa gcatcggcta actccgtgcc agcagccgcg gta
//


LOCUS       FJ595671              183 bp    DNA     linear   ENV 26-JAN-2009
DEFINITION  Uncultured bacterium clone DES-10 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   FJ595671
VERSION     FJ595671
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 183)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen conversion and bacterial diversity in sediment
            from shrimp pond under laboratory condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 183)
  AUTHORS   Kutako,M., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-DEC-2008) Graduate School, Chulalongkorn \
            University, 254 Phayathai Road, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..183
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="sediment"
                     /db_xref="taxon:77133"
                     /clone="DES-10"
                     /environmental_sample
     rRNA            <1..>183
                     /product="16S ribosomal RNA"
ORIGIN
        1 tgaccgtgcg aatatttgca ccatgggcgc aagcctgatg cagccatgcc gcgtgtatga
       61 agaaggcctt cgggttgtaa agtacttttc tcaatgagga aggcggatac gttaatagca
      121 atttcgtttg actttagctg cagaagaagc accggctaac tccgtgccag cagccgcggt
      181 aat
//
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LOCUS       FJ665393              163 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665393
VERSION     FJ665393
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 163)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
             in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 163)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..163
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-1"
                     /environmental_sample
     rRNA            <1..>163
                     /product="16S ribosomal RNA"
ORIGIN
        1 ggggaaaccc tgatccagcc atcccgcgtg agtgaagaag gccttcgggt tgtaaagctc
       61 tttcgaaagt gaagaaaact tatttcgtaa tatgttataa gtatgacggt aactttagaa
      121 gaagcaccgg ctaactacgt gccagcagcc gcggtaatta aaa
//


LOCUS       FJ665394              160 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-2 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665394
VERSION     FJ665394
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 160)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
            in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 160)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..160
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-2"
                     /environmental_sample
     rRNA            <1..>160
                     /product="16S ribosomal RNA"
ORIGIN
        1 ggaaaccctg atccagcaat gccgcgtgtg cgaacaaggc cttcgggttg taaagcactt
       61 ttgtttggaa agaaatcgtt tttgctaata ccaaagacgg atgacggtac caatcgaata
      121 agcaccggct aactacgtgc cagcagccgc ggtaattaaa
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LOCUS       FJ665395              162 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665395
VERSION     FJ665395
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 162)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
            in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 162)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..162
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-3"
                     /environmental_sample
     rRNA            <1..>162
                     /product="16S ribosomal RNA"
ORIGIN
        1 ggggaaaccc tgatgcagcc atgccgcgtg tgtgaagaag gccttcgggt tgtaaagcac
       61 tttcagtggt gaggaaggat gtaagattaa tactcttgca tattgacgtt agccacagaa
      121 gaagcaccgg ctaactccgt gccagcagcc gcggtaatta aa
//


LOCUS       FJ665396               141 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-4 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665396
VERSION     FJ665396
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 141)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
            in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 141)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..141
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-4"
                     /environmental_sample
     rRNA            <1..>141
                     /product="16S ribosomal RNA"
ORIGIN
        1 gggcgaaagc ctgacggagc aatctcccgt gagggatgac ggcctatggg ttgtaaacct
       61 ctttttttcag ggaggaataa atgacgtgta cctgaagaat aagcatcggc taactccgtg
      121 ccagcagccg cggtaataaa a
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LOCUS       FJ665397              163 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-5 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665397
VERSION     FJ665397
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 163)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
            in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 163)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..163
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-5"
                     /environmental_sample
     rRNA            <1..>163
                     /product="16S ribosomal RNA"
ORIGIN
        1 gcgcaagcct gatccagcca tgccgcgtgt gtgaagaagg ctttcgggtt gtaaagcact
       61 ttcagtgagg aggaaggcct taaggttaat acccttgagg attgacgtta ctcacagaag
      121 aagcaccggc taactccgtg ccagcagccg cggtaataaa aat
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LOCUS       FJ665398              155 bp    DNA     linear   ENV 11-FEB-2009
DEFINITION  Uncultured bacterium clone OUT-6 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   FJ665398
VERSION     FJ665398
KEYWORDS    ENV.
SOURCE      uncultured bacterium
  ORGANISM  uncultured bacterium
            Bacteria; environmental samples.
REFERENCE   1  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Changes in nitrogen compounds conversion and bacterial community
            in artificial shrimp pond under outdoor condition
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 155)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2009) Environmental Science, Chulalongkorn
            University, Pathumwan, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..155
                     /organism="uncultured bacterium"
                     /mol_type="genomic DNA"
                     /isolation_source="shrimp pond sediment"
                     /db_xref="taxon:77133"
                     /clone="OUT-6"
                     /environmental_sample
     rRNA            <1..>155
                     /product="16S ribosomal RNA"
ORIGIN
        1 gggagcctga tccagccatg ccgcgtgcag gaagactgcc ctatgggttg taaactgctt
       61 ttatacggga agaaacaccc cctcgtgagg gggcttgacg gtaccgtaag aataaggatc
      121 ggctaactcc gtgccagcag ccgcggtaat aaaaa
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LOCUS       FJ713782              176 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-1
ACCESSION   FJ713782
VERSION     FJ713782
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 176)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 176)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..176
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 tacactgatg cattcaacga gttccttgct tagacggctg ggtaagcttt tgaacttgaa
       61 tcgggatcgg gatagattat tattattgtt attcttgaac gaggaattcc aattccacgc
      121 aaatcctcat cctgcattgg ttacgtccct gcccttgccc ctcaccccaa ccgcan
//


LOCUS       FJ713793              177 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-2
ACCESSION   FJ713793
VERSION     FJ713793
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 177)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 177)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..177
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 tacactgatg catgcaacga gtatataacc ttggcctgaa aggcctgggt aatcttttga
       61 acgtgcatcg tgatagggat agattattgc aattattaat cttgaacgag gaattcctag
      121 taaacgcaaa tcatcaatct gcattgatta cgtccctgcc ctttgtacac accgaan
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LOCUS       FJ713794              179 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond Phy-3
ACCESSION   FJ713794
VERSION     FJ713794
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..179
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 acgcgcgcta cactgatgca ttcaacgagt tccttggccg agaggcctgg gtaatctttt
       61 gaacgtgcat cgtgataggg atagattatt gcaattatta atcttgaacg aggaattcct
      121 agtaaacgca gatcatcaat ctgcattgat tacgtccctg ccctttgtac acaccgcaa
//


LOCUS       FJ713795              181 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-4
ACCESSION   FJ713795
VERSION     FJ713795
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..181
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 tacactgatg cagtcagcga gcatactgta cctgcgccgg gaggcgtggg caatcttgtg
       61 aaactgcatc gtgctgggga tagatctttg taattatcga tcttgaacga ggaattccta
      121 gtaagcacga ttcatcagat cgtgttgatt acgtccctgc cctttgtaca caccgaannt
      181 n
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LOCUS       FJ713796              181 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-5
ACCESSION   FJ713796
VERSION     FJ713796
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 181)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 181)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..181
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 acgcgcgcta cactgatgaa gtcaacgagt attcaccttg gctggaaagt ctgggtaatc
       61 ttgtgaaact ttatcgtgct ggggatagtc acttgtaatt ttgacttgaa cgaggaattc
      121 ctagtaagcg tgagtcatca gctcgcgttg attacgtccc tgccctttgt acacaccgca
      181 a
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LOCUS       JF713797              191 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond. Band Phy-6
ACCESSION   JF713797
VERSION     JF713797
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 191)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 191)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
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       61 gggtaatctt gtgaaactgc atcgtgatgg ggcttgacga ttgcaattat ttgtcacaaa
      121 cgaggaattc ctagtaagcg caagtcatca acttgcattg attacgtccc tgccctttgt
      181 acacaccgca a
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LOCUS       FJ713798              335 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-7
ACCESSION   FJ713798
VERSION     FJ713798
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 335)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 335)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
     source          1..335
                     /organism="Eukaryotic microorganism from shrimp pond"
                     /mol_type="genomic DNA"
ORIGIN
        1 acgcgcgcta cactgatgaa gtcagcgagt tcactccttg gccgagaggt ctgggtaatc
       61 ttgtgaaact tcatcgtgct ggggatagac cattgtaatt attggtcttc aacgaggaat
      121 tcctagtaag cgcgagtcat cagctcgcgt tgattacgtc cctgcccttt gtacacaccg
      181 caaaaaaaag aaatatcccc ctcataagat ttttgtctac tacattggaa tattgctggt
      241 gatcctttcc atccttgtgg aagtacattg tactgatatc taatccctgg tgtctcattg
      301 tttatactag gtatggtgaa tgctgagtat tttct
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LOCUS       FJ713799              187 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-8
ACCESSION   FJ713799
VERSION     FJ713799
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 187)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 187)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
ORIGIN
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       61 aacccgttta atctccttcg tgctggggat tgaggtttgc aattgtcctt catgaacgag
      121 gaattcccag taagtgcaag tcataagctt gcgctgacta cgtccctgcc ctttgtacac
      181 accgcaa
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LOCUS       FJ713800              186 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-9
ACCESSION   FJ713800
VERSION     FJ713800
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
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REFERENCE   1  (bases 1 to 186)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 186)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
ORIGIN
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       61 acccccctgaa actccttcgt gcttgggatt gggacttgca actatttccc atgaacgagg
      121 aattcccagt aagtgcgggt cataagctcg cgttgattac gtccctgccc tttgtacaca
      181 ccgcaa
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LOCUS       FJ713801              179 bp    DNA     linear       06-FEB-2009
DEFINITION  Eukaryotic microorganism from shrimp pond, band Phy-10
ACCESSION   FJ713801
VERSION     FJ713801
KEYWORDS    .
SOURCE      Eukaryotic microorganism from shrimp pond
  ORGANISM  Eukaryotic microorganism from shrimp pond
            Unclassified.
REFERENCE   1  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Diversity of eukariotic microorganisms in an outdoor artificial
            shrimp pond
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 179)
  AUTHORS   Kutako,M., Limpiyakorn,T., Powtongsook,S. and Menasveta,P.
  CONSRTM   Chulalongkorn University
  TITLE     Direct Submission
  JOURNAL   Submitted (06-FEB-2009) Enviromental Science, Chulalongkorn
            University, Center of Excellence for Marine Biotechnology,
            Chulalongkorn University, Bangkok 10330, Thailand
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
ORIGIN
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       61 gaacccccctt cgtggtaggg atcggggctt gcaattattc cccgtgaacc aggaattccc
      121 agtaagcgca agtcataagc ttgcgttgat tacgtccctg ccctttgtac acaccgcaa
//
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BIOGRAPHY

Miss Maliwan Kutako was born on 14th November, 1977, in Sakolnakorn Province, Thailand. After completion of her primary and secondary education, she joined Burapha University, Chonburi Province, from here she received a B.Sc. degree in Biology in March, 2000. Thereafter, she continued study in Faculty of Science, King Mongkut's Institute of Technology Lardkrabang, with the scholarship from National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand. In February, 2004, she graduated a M.Sc. in Biotechnology. After graduation, she was a research assistant at the Center of Excellence for Marine Biotechnology (CEMB), Chulalongkorn University. In year 2005 to 2009, she studied a Ph.D. in Inter-department of Environmental Science, Chulalongkorn University and was awarded a Ph.D scholarship from the Cooperative Research Network grant, Bureau of Personnel Administration and Development, Ministry of Education, Royal Thai Government.

Research publications and presentations

Kutako, M., Yiewya, K., Nupasant, P., Ngamphongsai, C. and Powtongsook, S. 2005. Conversion of Inorganic Nitrogen Compounds from Organic Matter Decomposition in Sediment from Shrimp Pond under Laboratory Condition. In *Proc. 31st Congress on Science and Technology of Thailand*. Suranaree University of Technology, 18 – 20 October 2005. (Oral presentation)

Kutako, M., Jangrassa, S., Malaphol, A., Limpiyakorn, T., Powtongsook, S. and Menasveta, P. 2007. Changes of Inorganic Nitrogen Compounds and Bacterial Diversity in High Organic Content Sediment from Shrimp Pond. In *Proc. 33rd Congress on Science and Technology of Thailand*, Walailak University, Nakhonsithammarat, Thailand, October 18-20, 2007. (Oral presentation)

Kutako, M., Srisamrit, B., Arnthong, J., Powtongsook, S. and Menasveta, P. 2007. Use of Nitrification Biofilter on Nitrogen Treatment in Outdoor Aquaculture Tank. Journal of Environmental Research 29(2): 23-46.

Kutako, M., Saleedeang, A., Tapaneeyaworawong, P., Limpiyakorn, T., Powtongsook, S. and Menasveta, P. 2007. Effect of the Inorganic Carbon Addition on Nitrogen Conversion and Bacterial Diversity in the High Organic Content Sediment from Shrimp Pond. In *Proc. The 12th Biological Sciences Graduate Congress*, University of Malaya, Kuala Lumpur, Malaysia, December 17-18, 2007. (Oral presentation)

Kutako, M., Limpiyakorn, T., Powtongsook, S. and Menasveta, P. 2008. Evaluation of Nitrogen Compounds Conversion and Sediment Microbial in Pond Bottom Soil. *In Proc. The Science Forum 2008. Chulalongkorn University*, Bangkok, Thailand, March 13-14, 2008. (Oral presentation)

Kutako, M., Tapaneeyaworawong, P., Limpiyakorn, T., Powtongsook, S. and Menasveta, P. 2008. Effect of the Organic Carbon on Nitrogen Conversion and Sediment Bacterial Community in Aquaculture Soil Bottom. In *Proc. World Aquaculture 2008*. Busan, Korea, May 19-23, 2008. (Oral presentation)

Kutako, M., Limpiyakorn, T., Powtongsook, S. and Menasveta, P. 2008. Effect of Dissolved Oxygen on Nitrogen Conversion and Sediment Bacterial Community in Aquaculture Bottom Soil under Laboratory Condition. In *Proc. 2008 Forum on Fishery Science and Technology: A Serial Forum of Engineering and Technology of Chinese Academy of Engineering*, Shanghai, China, September 26-28, 2008. (Oral presentation)

Kutako, M., Somkhiawwan, S., Permpool, B., Donnuea, S. Limpiyakorn, T. Powtongsook, S. and Menasveta, P. 2008. Effect of Illumination on Inorganic Nitrogen Conversion of Sediment from Shrimp Pond under Laboratory Condition. In *Proc. 34th Congress on Science and Technology of Thailand*, Queen Sirikit National Convention Center, Bangkok, Thailand, October 31-November 1, 2008. (Oral presentation)